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(54) Title: METHODS OF TREATMENT OF WASTING SYNDROME BASED ON ADMINISTRATION OF DERIVATIVES OF HUMAN CHORIONIC GONADOTROPIN

(57) Abstract

The present invention relates to methods of treating or preventing wasting syndrome by administration of human chorionic gonadotropin, β-human chorionic gonadotropin, a peptide containing a sequence of a portion of β-human chorionic gonadotropin, or a fraction of a source of native human chorionic gonadotropin or β-human chorionic gonadotropin. In a preferred embodiment, the invention relates to β-human chorionic gonadotropin peptides for treatment or prevention of wasting syndrome. The invention further provides assays for the utility of particular human chorionic gonadotropin preparations in the treatment or prevention of wasting syndrome. Pharmaceutical compositions and methods of administration are also provided.

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METHODS OF TREATMENT OF WASTING SYNDROME BASED ON ADMINISTRATION OF DERIVATIVES OF HUMAN CHORIONIC GONADOTROPIN

1. CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation in part of co-pending application Serial No. 08/709,933, filed September 9, 1996, which is a continuation in part of Serial No. 08/669,675, filed June 24, 1996 now abandoned, both of which are incorporated by reference herein in their entireties.

2. FIELD OF THE INVENTION

The present invention is directed to methods of treatment and prevention of wasting syndromes by administration of human chorionic gonadotropin preparations, fractions of human chorionic gonadotropin preparations, the β-chain of human chorionic gonadotropin and peptides containing a sequence of a portion or portions of the β-chain of human chorionic gonadotropin. The invention also provides pharmaceutical compositions comprising human chorionic gonadotropin preparations, fractions of human chorionic gonadotropin preparations, the β-chain of human chorionic gonadotropin or peptides having a sequence of a portion or portions of the β-chain of human chorionic gonadotropin.

3. BACKGROUND OF THE INVENTION

3.1. WASTING SYNDROMES

Wasting syndrome is a serious clinical problem characterized by a decrease in body mass of more than 10% from baseline body weight and a disproportionate loss of body mass with respect to body fat (Weinroth et al., 1995, *Infectious Agents and Disease* 4:76-94; Kotler and Grunfeld, 1995, *AIDS Clin. Rev.* 96:229-275). Thus, wasting is distinguished from starvation in which higher levels of body fat than body cell mass are depleted (Kotler et al., 1985, *Am J. Clin. Nutr.* 42:1255-1265; Cahill, 1970, *N. Engl. J. Med.* 282:668-675). Wasting is associated with a variety of conditions, including HIV infection (human immunodeficiency virus (HIV) has been implicated in acquired immune deficiency

syndrome (AIDS) (Barre-Sinoussi, F., et al., 1983, *Science* 220:868-870; Gallo, R., et al., 1984, *Science* 224:500-503)), other infectious diseases, sepsis, cancer, chronic cardiovascular disease and diarrhea (Kotler et al., 1989, *Am. J. Clin. Nutr.* 50:444-447; Heymsfield et al., 1982, *Am. J. Clin. Nutr.* 36:680-690). Importantly, wasting is a significant factor in the mortality of patients suffering from infections or cancer. In fact, body cell mass depletion has a linear relationship to time of survival in AIDS patients (Kotler et al., 1989, *Am. J. Clin. Nutr.* 50:444-447).

The cause of wasting syndrome in AIDS and other conditions is unclear and is most likely multifactorial. Metabolic abnormalities, irregular levels of hormones and cytokines, and malabsorption have all been implicated in wasting syndrome. Not all AIDS patients suffer from wasting, suggesting that the cause of the wasting is not HIV itself. Most cases of HIV associated wasting syndrome are apparently caused by complications of AIDS, such as secondary infections and gastrointestinal disease (Kotler and Grunfeld, 1995, *AIDS Clin. Rev.* 96:229-275).

Current and potential therapies for wasting syndromes include nutritional support, appetite enhancers such as dronabinol and megestrol acetate, anabolic therapies, such as growth hormone, and cytokine inhibitors. However, mixed results have been obtained with nutritional support and appetite enhancers in that patients tended to gain only fat and not overall body mass. Administration of growth hormone, and cytokine inhibitors are still being tested and may pose a risk of side effects (Kotler and Grunfeld, 1995, *AIDS Clin. Rev.* 96:229-275; Weinroth et al., 1995, *Infectious Agents and Disease* 4:76-94).

Thus, treatment of wasting is critical to the survival and well-being of patients suffering from serious diseases such as cancer and AIDS; thus, there is a need for safe and effective therapies for wasting syndrome associated with cancer, AIDS and other infectious diseases.

3.2. HUMAN CHORIONIC GONADOTROPIN

Human chorionic gonadotropin (hCG), which is required for the maintenance of pregnancy, is a member of the glycoprotein hormone family. The glycoprotein hormones, which also include follicle-stimulating hormone (FSH), luteinizing hormone (LH) and thyroid-stimulating hormone (TSH), consist of two sub-units, α and β. These subunits are non-covalently linked to form a heterodimer, and heterodimer formation has been shown to be required for receptor binding. Within a particular species, the α-subunits are identical among the glycoprotein hormones while the β-subunits differ and determine the receptor binding specificity of the particular hormone (Kornyei, J.L., et al., 1993, *Biol. Reprod.* 49:1149). The β-subunits of the glycoprotein hormones exhibit a high degree of sequence similarity within the N-terminal 114 amino acids. LH is the most similar to hCG with 85% sequence homology within the first 114 amino acids, and both proteins bind the same receptor. hCG, however, contains a C-terminal extension not present in the other glycoprotein β-chains (Lapthorn, A.J., et al., 1994, *Science* 369:455-461).

From the three dimensional crystal structure of hCG, it was determined that hCG, like the growth factors nerve growth factor (NGF), transforming growth factor-β (TGF-β) and platelet-derived growth factor-β (PDGF-β), is a cysteine-knot glycoprotein. Proteins containing such a cysteine-knot motif have at least three disulfide bridges, two of which join adjacent anti-parallel strands of the peptide, thus, forming a ring, and one of which joins the peptide chain through the ring. Particular structures in the hCG β-chain include the determinant loop sequence (β93-100) which has been implicated in subunit association and the longest inter-cysteine loop (β38-57) which may play a role in receptor binding. Residues 47-53 appear to be exposed at the surface of this inter-cysteine loop (Lapthorn et al., 1994, *Nature* 369:455-461).

Harris, P.J. (1995, *The Lancet* 346:118-119) found that administration of an hCG preparation to certain HIV infected

patients with hypogonadotropic hypogonadism, among other improvements in symptoms of AIDS, resulted in an improvement in wasting.

The present invention fulfills a need for safe and effective therapies for wasting syndrome associated with AIDS, other infectious diseases and cancer.

Citation of references hereinabove shall not be construed as an admission that such references are prior art to the present invention.

4. SUMMARY OF THE INVENTION

The present invention further relates to therapeutic methods and compositions for treatment and prevention of wasting syndromes based on hCG and β-hCG preparations, therapeutically and prophylactically effective fractions of a source of native hCG or native β-hCG and therapeutically and prophylactically effective proteins containing a sequence of a portion or portions (*i.e.*, a fusion protein comprising more than one β-hCG peptide sequence, *e.g.*, having an amino acid sequence of one β-hCG peptide linked via a peptide bond to another β-hCG peptide) of β-hCG, and related derivatives and analogs. The invention provides for treatment and prevention of wasting syndromes by administration of a therapeutic compound of the invention. The therapeutic compounds of the invention include: hCG, β-hCG, therapeutically and prophylactically effective fractions of a source of hCG or β-hCG (preferably a source of native hCG or native β-hCG, *i.e.* a source of naturally occurring hCG or β-hCG and not recombinantly produced hCG or β-hCG), therapeutically and prophylactically effective peptides having a sequence of a portion or portions of β-hCG (*i.e.* a fusion protein comprising more than one β-hCG peptide sequence either as non-contiguous or contiguous sequences, *e.g.* having an amino acid sequence of one β-hCG peptide linked via a peptide bond to another β-hCG peptide), modified derivatives of hCG, β-hCG and β-hCG peptides, and nucleic acids encoding β-hCG and therapeutically and prophylactically effective peptides

having a sequence of a portion or portions of β-hCG, and derivatives and analogs of the foregoing.

The present invention also relates to the use of certain fractions (*i.e.* components of a source of hCG or β-hCG isolated away from other components in the source of hCG or β-hCG by a separation technique known in the art) of any source of hCG or β-hCG, such as commercial hCG preparations and human (preferably early, *i.e.*, first trimester) pregnancy urine, which fractions have anti-HIV and/or anti-Kaposi's Sarcoma activity and/or anti-wasting activity.

The invention also provides *in vitro* and *in vivo* assays for assessing the efficacy of therapeutics of the invention for treatment or prevention of wasting syndromes.

The invention also provides pharmaceutical compositions and methods of administration of Therapeutics of the invention for treatment.

5. DESCRIPTION OF THE FIGURES

Figures 1A-E. Effects of an hCG preparation, APL™ (Wyeth-Ayerst), β-hCG peptides and certain fractions of hCG APL™ and early preganancy urine on weight and HIV-1 gene expression in HIV-1 transgenic mice. (A) and (C) Weight change in grams in individual HIV-1 transgenic mice after treatment from from day 1 to day 10 post partum is represented as a bar graph with open bars representing the weight at day 1 and solid bars representing the weight at day 10. (B), (D) and (E) Suppression of HIV-1 gene expression in transgenic mice. The bar graph presents the level of expression in pixels, as determined by chemiluminescence assay of the HIV genes env, tat, rev, and nef in the individual HIV transgenic mice. For (B) and (D), the black bars represent tat expression, the striped bars represent rev expression, the lightly stippled bars represent env expression, the open bars represent nef expression. For (E), the striped bars represent env expression, the solid bars represent rev expression, and the open bars represent nef expression. In (A) and (B), bars 1-3 represent untreated

control transgenic mice; bars 4-6 represent mice whose mothers received (subjects were administered through the mothers' milk) 200 μg circularized β-hCG peptide 44-57 (with cysteine substituted at position 44; SEQ ID NO:26) per day; bars 7-9 represent mice whose mothers received 300 IU per day hCG-APL™; and bars 9-11 represent mice whose mothers received 200 μg per day of the fused β-hCG peptide 45-57::109-119 (SEQ ID NO:30). In (C) and (D), the bars labeled "untreated" represent mice receiving only PBS; and the bars labeled "treated" or "treated with HAFc" represent mice administered 300 IU per day of hCG APL™. In (E), the set of bars labeled "1" represents mice treated with PBS alone; "2" represents mice treated with 100 μg β-hCG core peptide per day; "3" represents mice treated with 100 μg per day α-hCG; "4" represents mice treated with 200 μl per day of fraction 61 of the hCG APL™ fractionation; and "5" represents mice treated with 200 μl per day of fraction 65 of the early pregnancy urine fractionation.

Figures 2A-D. Effects of an hCG preparation on indicators of SIV infection in SIV-infected macaques. SIV was given intravenously at a dose of $10^{4.5}$ $TCID_{50}$ per ml. (A) SIV titer was monitored over time in months by quantifying the p27 gag protein (Organon Teknika assay) as nanograms (ng) of p27/ml of plasma from the plasma of the SIV infected macaques. Treated SIV-infected macaques (indicated as Rx) were given hCG APL, 3000 IU, 2x weekly. Plasma levels of p27 gag in these treated monkeys are indicated on the graph by lines with diamonds, number (#) signs or filled circles. Results with the untreated SIV-infected macaques (indicated UnRx) are indicated by the lines with either stars or triangles. (B) $CD4^+$ T cell levels were determined in cells/mm^3 in SIV-infected macaques either treated with hCG or untreated over time in months. Results from the SIV-infected monkeys treated with hCG (APL) (Rx) are indicated by lines with diamonds, number (#) signs or filled circles, while results with the untreated monkeys (UnRx) are indicated by lines with stars or triangles. (C) Change in weight in

kilograms (kg) was monitored in treated and untreated SIV-infected monkeys over time in months. Weight changes in the SIV-infected monkeys treated with hCG (APL) (Rx) are indicated by lines with diamonds, # signs or filled circles, while results in the untreated monkeys (UnRx) are indicated by lines with stars or triangles. (D) Levels of $CD4^+$ T cells were monitored in normal uninfected monkeys either treated with hCG (APL) or untreated over time in months. $CD4^+$ T cell levels in the untreated monkeys are indicated by lines with sun-like figures or squares, and the results in the treated monkeys are indicated by lines with pentagonal figures or with filled inverted triangles.

Figures 3A-J. Effects of administration of hCG preparations on HIV-1 viral load and $CD4^+$ T cell levels in individual patients in the clinical study described in Section 7.3 *infra*. Figures A and B are data from patient PHOJ, C and D from patient PG1, E and F from patient PG3, G and H from patient PHVE, and I and J from patient PG17. In panels A, C, E, G and I, viral load and $CD4^+$ T Cell counts are plotted over time (in months). Viral load (measured by RT-PCR in panels A and G and by the Roche Amplicor test in panels C, E and I) is plotted as the logarithm of the viral load (represented by line with "X" data points). The $CD4^+$ T Cell levels are plotted as $CD4^+$ T Cells/ml (represented by line with triangle data points). Panels B, D, F, H, and J plot the dosage of hCG in IU (X 1000) per week over time in months, with the timing of other therapies indicated above the graph with a thick arrow.

Figure 4. The nucleotide (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequences of β-hCG.

Figures 5A and B. Schematic depiction of the structures of (A) the linear peptide of amino acids 45-57 (SEQ ID NO:6) of the β-hCG sequence depicted in Figure 4 (SEQ ID NO:2) where the amino acid residues at positions 47 and 51 are substituted by a branch made up of diaminobutyric acid peptide bonded to proline, and (B) the circularized peptide of amino acids 44-57 (SEQ ID NO:12) with valine at position

44 substituted with cysteine, which protein is circularized via a disulfide bond between its amino- and carboxy-terminal cysteines. In both A and B, amino acids are represented by their three letter amino acid code, except for the branched residues and the terminal cysteines, for which the structure is depicted.

Figures 6A-F. These graphs depict results from the fractionation by SUPERDEX™ 200 gel filtration of a commercial hCG preparation APL™ (Wyeth Ayerst) and early pregnancy urine. (A) and (D). These graphs depicts the relative amount of protein in mg/ml in each fraction identified by fraction number in the hCG APL™ fractionation (A) and early pregnancy urine fractionation (D). The fractions containing the hCG dimer and β-core protein are identified with arrows and the labels "hCG" and "β-core" respectively. (B) and (E). These graphs present the percent inhibition of growth of cultured KS cells by the individual fractions from the hCG APL™ (B) and early pregnancy urine (E) using KS cell clonogenic assays. The results are plotted as percent inhibition versus fraction number. (C) and (F). These graphs depict the effect of the hCG APL™ (C) and early pregnancy urine (F) fractions on HIV replication in vitro. Specifically, this graph presents data on the percentage inhibition of HIV-1 IIIB viral infection of PBMCs as a function of fraction number.

Figures 7A-C. Graphs of change in viral load and $CD4^+$ T cell levels with hCG therapy. (A) The change in viral load is plotted as the logarithm of viral load after therapy ("Logload") as a function of viral load before therapy ("Baselog"). (B) The change in $CD4^+$ T cell levels is plotted as $CD4^+$ T cell levels after therapy (in $CD4^+$ T cells/ml) ("CD4") as a function of $CD4^+$ T cell levels before therapy (in $CD4^+$ T cells/ml) ("CD4Base"). (C) Plot of linear regression analysis of the change in viral load ("vlchange") as a function of weekly dose of hCG in IU ("HCGIU"). For all three panels, data points for patients on hCG therapy as well as non-protease and protease inhibitors are represented by open triangles, those on hCG therapy and non-protease

inhibitors by open diamonds, and those on hCG alone by solid circles.

Figures 8A and B. (A) Plot of protein concentration (as mAUFS, milli absorbance units, at 280 nm) as a function of the fraction number of the hCG APL™ preparation SUPERDEX™ 200 fractionation. (B) Plot of protein concentration (as mAUFS, milli absorbance units, at 280 nm) of molecular weight markers of 670 kD, 158 kD, 44 kD, 17 kD and 1.3 kD (as indicated above the plot) as a function of fraction number of a SUPERDEX™ 200 column run under the same conditions as the fractionation plotted in panel A.

Figures 9A-E. Mass spectrometry profiles of fractions 61, 63, 64, 65, and 67 in panels A-E, respectively.

6. <u>DETAILED DESCRIPTION OF THE INVENTION</u>

The present invention further relates to therapeutic methods and compositions for treatment and prevention of wasting syndromes based on hCG and β-hCG preparations, therapeutically and prophylactically effective fractions of a source of hCG or β-hCG, and therapeutically and prophylactically effective β-hCG peptides. The invention provides for treatment of wasting syndrome by administration of a therapeutic compound (termed herein "Therapeutic") of the invention. Such Therapeutics of the invention include but are not limited to: hCG, β-hCG, therapeutically and prophylactically effective fractions of a source of native hCG or native β-hCG, therapeutically and prophylactically effective β-hCG proteins (*i.e.*, those peptides which prevent or treat wasting syndrome), related derivatives and analogs of hCG, β-hCG or β-hCG peptides, and nucleic acids encoding β-hCG and β-hCG peptides, and analogs and derivatives thereof. β-hCG peptides which are effective for treatment and prevention of wasting syndromes can be identified by *in vitro* and *in vivo* assays such as those described in Section 6.3, *infra*.

In a preferred embodiment, a therapeutic composition of the invention comprises a β-hCG peptide, the amino acid

sequence of which consists of amino acid numbers 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, or 48-56 (SEQ ID NOS:8-25 or 33-35, respectively) of Figure 4 (a portion of SEQ ID NO:2), particularly a β-hCG peptide which consists of amino acid numbers 41-54, 45-54 or 109-119 (SEQ ID NOS:3, 4, or 7, respectively), most preferably of a β-hCG peptide which consists of amino acid numbers 47-53 (SEQ ID NO:5) or 45-57 (SEQ ID NO:6). In another preferred embodiment, a therapeutic composition of the invention comprises a fusion protein comprising more than one β-hCG sequence, *e.g.* having an amino acid sequence of one β-hCG peptide linked via a peptide bond to another β-hCG peptide, in particular a protein, the amino acid sequence of which consists of amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of amino acids 110-119 (SEQ ID NO:27); or an isolated protein of amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 108-119 (SEQ ID NO:29) of the β-hCG sequence depicted in Figure 4 (portions of SEQ ID NO:2), *i.e.*, the peptides denoted 45-57::109-119, 110-119::45-57, or 47-57::108-119 (SEQ ID NOS:30-32, respectively).

In other preferred embodiments, the therapeutic comprises a β-hCG peptide, the amino acid sequence of which consists of circularized (via a disulfide bond between its amino- and carboxy-terminal cysteines) 44-57 (SEQ ID NO:26) with the valine at position 44 substituted with cysteine ((Val44Cys) 45-57 circularized) (depicted in Figure 5B), the circularized (via a disulfide bond between its amino- and carboxy- terminal cysteines) fused peptide of amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus by a peptide bond to the N-terminus of amino acids 45-57 (SEQ ID NO:6), or the peptide 45-57 (SEQ ID NO:6) where the amino acid residues at positions 47 and 51 are substituted by a branch, where the

branches are made up of diaminobutyric acid peptide bonded to a proline residue (depicted in Figure 5A). The amino acid sequence of β-hCG is depicted in Figure 4 (SEQ ID NO:2).

In yet another embodiment, the therapeutic comprises fractions, preferably fractions of a source of hCG or β-hCG, such as commercial hCG preparations and human pregnancy urine (preferably early, *i.e.* first trimester), of material eluting from a gel filtration column, preferably a SUPERDEX 200™ column, with apparent molecular weights of approximately 40 kD, 15 kD or 2-3 kD as determined based on in which fractions compared native hCG dimer (77kD) and β-hCG core (10 kD) elute.

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections which follow.

6.1. THERAPEUTIC USES

The invention provides for treatment or prevention of wasting syndrome by administration of a therapeutic compound (termed herein "Therapeutic"). Such "Therapeutics" include, but are not limited to: hCG, β-hCG, therapeutically and prophylactically effective fractions of a source of native hCG or native β-hCG (*i.e.* naturally occurring hCG or β-hCG and not recombinantly produced hCG or β-hCG), and therapeutically and prophylactically effective β-hCG peptides, *i.e.*, those fractions and peptides which prevent or treat wasting syndrome (*e.g.*, as demonstrated in *in vitro* and *in vivo* assays described *infra*), and derivatives and analogs thereof, as well as nucleic acids encoding hCG, β-hCG and therapeutically and prophylactically effective β-hCG peptides and derivatives and analogs thereof (*e.g.*, for use in gene therapy).

The methods of the invention can be used for treatment or prevention of any disease or disorder characterized by a loss of body cell mass. Particular conditions that can be treated by methods of the invention include, but are not limited to, wasting associated with viral, such as HIV,

bacterial or other types of infections, and sepsis; cachexia associated with cancer, chemotherapy, and radiation therapy; wasting associated with chronic cardiovascular disease; wasting caused by exposure to toxic substances; wasting associated with diarrhea and other gastrointestinal disorders.

In a preferred embodiment, a Therapeutic of the invention is administered to treat or prevent a wasting syndrome associated with HIV infection. In another preferred embodiment, a Therapeutic of the invention is administered to treat or prevent a wasting syndrome associated with cancer.

6.2. THERAPEUTICS OF THE INVENTION

6.2.1. hCG, β-hCG AND β-hCG PEPTIDES AND DERIVATIVES

Native preparations of hCG and β-hCG can be obtained from a variety of sources. Both hCG and β-hCG are commercially available (*e.g.*, Sigma Chemical Company) and hCG is commercially available in a form suitable for Therapeutic use in humans (*e.g.*, from Fujisawa, Wyeth-Ayerst Laboratories (APL™), Organon, Inc. (Pregnyl™) and Serono Laboratories, Inc. (Profasi™)). The inventors have discovered that different sources of hCG have variable effects on wasting; thus, one aspect of the invention relates to assaying preparations of hCG for efficacy in treatment or prevention of wasting syndrome. The therapeutic effectiveness of hCG preparations can be tested by the assays in animal models described in Section 6.3 *infra* or by any method known in the art. It is preferable to test the hCG preparation in an animal model, such as HIV-1 transgenic mice or SIV infected monkeys, before testing the preparation in humans.

In a specific embodiment, a preparation comprising hCG is used that contains not only the hCG heterodimer but also peptide fragments thereof, *e.g.*, β chain peptides.

hCG and β-hCG can also be purified, or preferably partially purified, from any source known to contain hCG, *e.g.*, urine from pregnant women, using conventional techniques well-known in the art, such as affinity

chromatography. For example, antibodies prepared against hCG or β-hCG can be used to prepare an affinity chromatography column which can be used to purify the proteins by well-known techniques (*see, e.g.*, Hudson & May, 1986, *Practical Immunology*, Blackwell Scientific Publications, Oxford, United Kingdom).

The β-hCG-related proteins are preferably prepared by any chemical or enzymatic synthesis method known in the art, as described *infra*.

In a preferred embodiment of the invention, proteins (*e.g.*, peptides) and derivatives, the amino acid sequence of which consists of a portion of the β-hCG sequence (β-hCG peptides), and derivatives thereof, are used to treat or prevent wasting syndrome. In various specific embodiments, the portions of the β-hCG sequence are at least 3, 5, 10, 20, or 30 amino acids. These peptides are preferably β-hCG peptides, or nucleic acids encoding β-hCG peptides, from amino acids 41-54, 45-54, 47-53 and 45-57 (SEQ ID NOS:3-6, respectively) of Figure 4 (a portion of SEQ ID NO:2). In other embodiments, β-hCG peptides of 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, and 48-56 (SEQ ID NOS:7-25 and 33-35, respectively) of Figure 4 (a portion of SEQ ID NO:2) are used to treat or prevent wasting syndrome.

In another embodiment, a protein is used which contains the amino acid sequence of two or more at least 5, 7 or 10 amino acid, non-naturally contiguous portions of the β-hCG sequence (Figure 4 (SEQ ID NO:2)) linked by peptide bonds between the N-terminus of one portion and the C-terminus of another portion. In a specific embodiment, a protein is used, the amino acid sequence of which consists of amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of amino acids 110-119 (SEQ ID NO:27); or a protein is used that has an amino acid sequence of amino

acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 108-119 (SEQ ID NO:29) of the β-hCG sequence depicted in Figure 4 (portions of SEQ ID NO:2); *i.e.*, the fused peptides represented as 45-57::109-119, 110-119::45-57, or 47-57::108-119 (SEQ ID NOS:30-32, respectively). Derivatives of the foregoing fusion proteins are also provided (*e.g.*, branched, cyclized, N- or C-terminal chemically modified, etc.). In another embodiment, fusion proteins comprising two or more such portions of the β-hCG sequence are provided; such portions may or may not be contiguous to one another (*i.e.*, an intervening sequence may be present). Molecules comprising such portions linked by hydrocarbon linkages are also provided.

In another embodiment, the peptides of the invention (i) have an amino acid sequence consisting of no more than 8 peptides of the β-hCG sequence (Figure 4 (SEQ ID NO:2)) and (ii) comprise amino acid numbers 47-53 (SEQ ID NO:5) of β-hCG (Figure 4 (SEQ ID NO:2)). In another embodiment, the invention provides an isolated protein which protein (a) comprises one or more portions of the amino acid sequence of β-hCG, a peptide consisting of said portion(s) being effective to inhibit HIV infection or replication; and (b) lacks β-hCG amino acids contiguous to said portion(s). In a specific embodiment, the invention provides an isolated protein (a) comprising a β-hCG amino acid sequence consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 45-57, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-57, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, and 48-56 (SEQ ID NOS:3-25 and 33-35 respectively), as depicted in Figure 4 (a portion of SEQ ID NO:2); and (b) lacking β-hCG amino acids contiguous to said sequence. Peptides containing the above sequences in which only conservative substitutions have been made are also provided by the present invention, as but one example of peptide derivatives within the scope of the invention. Analogs of the above-mentioned proteins and

peptides which have one or more amino acid substitutions forming a branched peptide (*e.g.*, by substitution with an amino acid or amino acid analog having a free amino- or carboxy-side chain that forms a peptide bond with a sequence of one or more amino acids, including but not limited to prolines) or allowing circularization of the peptide (*e.g.*, by substitution with a cysteine, or insertion of a cysteine at the amino- or carboxy-terminus or internally), to provide a sulfhydryl group for disulfide bond formation, are also provided. Other β-hCG peptides, and nucleic acids encoding these peptides, may have utility in the therapeutic methods of the invention. The utility of β-hCG peptides may be determined by the *in vitro* and *in vivo* assays described in Section 6.3 *infra* or by any other method known in the art.

In specific embodiments, peptides of less than 50, or less than 25, amino acids are provided.

The invention also relates to derivatives, modifications and analogs of β-hCG peptides. In a specific embodiment, a purified derivative of a protein, the amino acid sequence of which protein is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 or 48-56 (SEQ ID NOS:3-25 or 33-35, respectively) as depicted in Figure 4 (a portion of SEQ ID NO:2) is used to treat or prevent wasting syndrome. In one embodiment, β-hCG peptide derivatives can be made by altering the β-hCG peptide sequence by substitutions, additions or deletions that provide for therapeutically effective molecules. Thus, the β-hCG peptide derivatives include peptides containing, as a primary amino acid sequence, all or part of the particular β-hCG peptide sequence including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a peptide which is functionally active. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional

equivalent, resulting in a silent alteration. Conservative substitutions for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Such β-hCG peptide derivatives can be made either by chemical peptide synthesis or by recombinant production from a nucleic acid encoding the β-hCG peptide which nucleic acid has been mutated. Any technique for mutagenesis known in the art can be used, including but not limited to, chemical mutagenesis, *in vitro* site-directed mutagenesis (Hutchinson, C., et al., 1978, *J. Biol. Chem* 253:6551), use of TAB® linkers (Pharmacia), etc.

In addition, β-hCG peptides and analogs and derivatives of β-hCG peptides can be chemically synthesized. (*See, e.g.*, Merrifield, 1963, *J. Amer. Chem. Soc.* 85:2149-2156.) For example, peptides can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography (*e.g.*, *see* Creighton, 1983, Proteins, Structures and Molecular Principles, W.H. Freeman and Co., N.Y., pp. 50-60). β-hCG peptides can also be synthesized by use of a peptide synthesizer. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; *see* Creighton, 1983, Proteins, Structures and Molecular Principles, W.H. Freeman and Co., N.Y., pp. 34-49). Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the β-hCG peptide. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, α-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino

butyric acid, γ-Abu, ε-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, Nα-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

By way of example but not by way of limitation, peptides of the invention can be chemically synthesized and purified as follows: Peptides can be synthesized by employing the N-α-9-fluorenylmethyloxycarbonyl or Fmoc solid phase peptide synthesis chemistry using a Rainin Symphony Multiplex Peptide Synthesizer. The standard cycle used for coupling of an amino acid to the peptide-resin growing chain generally includes: (1) washing the peptide-resin three times for 30 seconds with N,N-dimethylformamide (DMF); (2) removing the Fmoc protective group on the amino terminus by deprotection with 20% piperidine in DMF by two washes for 15 minutes each, during which process mixing is effected by bubbling nitrogen through the reaction vessel for one second every 10 seconds to prevent peptide-resin settling; (3) washing the peptide-resin three times for 30 seconds with DMF; (4) coupling the amino acid to the peptide resin by addition of equal volumes of a 250 mM solution of the Fmoc derivative of the appropriate amino acid and an activator mix consisting or 400 mM N-methylmorpholine and 250 mM (2-(1H-benzotriazol-1-4))-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) in DMF; (5) allowing the solution to mix for 45 minutes; and (6) washing the peptide-resin three times for 30 seconds of DMF. This cycle can be repeated as necessary with the appropriate amino acids in sequence to produce the desired peptide. Exceptions to this cycle program are amino acid couplings predicted to be difficult by nature of their hydrophobicity or predicted inclusion within a helical formation during synthesis. For these situations, the above cycle can be

modified by repeating step 4 a second time immediately upon completion of the first 45 minute coupling step to "double couple" the amino acid of interest. Additionally, in the first coupling step in peptide synthesis, the resin can be allowed to swell for more efficient coupling by increasing the time of mixing in the initial DMF washes to three 15 minute washes rather than three 30 second washes. After peptide synthesis, the peptide can be cleaved from the resin as follows: (1) washing the peptide-resin three times for 30 seconds with DMF; (2) removing the Fmoc protective group on the amino terminus by washing two times for 15 minutes in 20% piperdine in DMF; (3) washing the peptide-resin three times for 30 seconds with DMF; and (4) mixing a cleavage cocktail consisting of 95% trifluoroacetic acid (TFA), 2.4% water, 2.4% phenol, and 0.2% triisopropysilane with the peptide-resin for two hours, then filtering the peptide in the cleavage cocktail away from the resin, and precipitating the peptide out of solution by addition of two volumes of ethyl ether. To isolate the peptide, the ether-peptide solution can be allowed to sit at -20°C for 20 minutes, then centrifuged at 6,000xG for 5 minutes to pellet the peptide, and the peptide can be washed three times with ethyl ether to remove residual cleavage cocktail ingredients. The final peptide product can be purified by reversed phase high pressure liquid chromatography (RP-HPLC) with the primary solvent consisting of 0.1% TFA and the eluting buffer consisting of 80% acetonitrile and 0.1% TFA. The purified peptide can then be lyophilized to a powder.

In a preferred embodiment, the invention provides a peptide with branched amino acids (branched peptide), preferably a branched peptide of amino acids 45-57 (SEQ ID NO:6) with branches occurring at positions 47 and 51, respectively, instead of the Gly and Ala residues normally present. Most preferably, diaminobutyric acid is substituted for the gly and ala residues at positions 47 and 51, respectively, and proline bonded to both diaminobutyric acid residues (45-57 branched) as shown in Figure 5A.

In other specific embodiments, branched versions of the β-hCG peptides listed hereinabove are provided, *e.g.*, by substituting one or more amino acids within the β-hCG sequence with an amino acid or amino acid analog with a free side chain capable of forming a peptide bond with one or more amino acids (and thus capable of forming a "branch").

Branched peptides may be prepared by any method known in the art for covalently linking any naturally occurring or synthetic amino acid to any naturally occurring or synthetic amino acid in a peptide chain which has a side chain group able to react with the amino or carboxyl group on the amino acids so as to become covalently attached to the peptide chain. In particular, amino acids with a free amino side chain group, such as, but not limited to, diaminobutyric acid, lysine, arginine, ornithine, diaminopropionic acid and citrulline, can be incorporated into a peptide so that an amino acid can form a branch therewith, for example, by forming a peptide bond to the free amino side group, from that residue. Alternatively, amino acids with a free carboxyl side chain group, such as, but not limited to, glutamic acid, aspartic acid and homocitrulline, can be incorporated into the peptide so that an amino acid can form a branch therewith, for example, by forming a peptide bond to the free carboxyl side group, from that residue. The amino acid forming the branch can be linked to a side chain group of an amino acid in the peptide chain by any type of covalent bond, including, but not limited to, peptide bonds, ester bonds and disulfide bonds. In a specific embodiment, amino acids, such as those described above, that are capable of forming a branch point, are substituted for β-hCG residues within a peptide having a β-hCG sequence.

Branched peptides can be prepared by any method known in the art. For example, but not by way of limitation, branched peptides can be prepared as follows: (1) the amino acid to be branched from the main peptide chain can be purchased as an N-α-tert-butyloxycarbonyl (Boc) protected amino acid pentafluorophenyl (Opfp) ester and the residue within the

main chain to which this branched amino acid will be attached can be an N-Fmoc-α-γ-diaminobutyric acid; (2) the coupling of the Boc protected amino acid to diaminobutyric acid can be achieved by adding 5 grams of each precursor to a flask containing 150 ml DMF, along with 2.25 ml pyridine and 50 mg dimethylaminopyridine and allowing the solution to mix for 24 hours; (3) the peptide can then be extracted from the 150 ml coupling reaction by mixing the reaction with 400 ml dichlormethane (DCM) and 200 ml 0.12N HCl in a 1 liter separatory funnel, and allowing the phases to separate, saving the bottom aqueous layer and re-extracting the top layer two more times with 200 ml 0.12 N HCl; (4) the solution containing the peptide can be dehydrated by adding 2-5 grams magnesium sulfate, filtering out the magnesium sulfate, and evaporating the remaining solution to a volume of about 2-5 ml; (5) the dipeptide can then be precipitated by addition of ethyl acetate and then 2 volumes of hexanes and then collected by filtration and washed two times with cold hexanes; and (6) the resulting filtrate can be lyophilized to achieve a light powder form of the desired dipeptide. Branched peptides prepared by this method will have a substitution of diaminobutyric acid at the amino acid position which is branched. Branched peptides containing an amino acid or amino acid analog substitution other than diaminobutyric acid can be prepared analogously to the procedure described above, using the N-F-moc coupled form of the amino acid or amino acid analog.

In a preferred embodiment, the peptide is a cyclic peptide, preferably a cyclic peptide of β-hCG amino acids 44-57 (SEQ ID NO:26) with cysteine substituted for valine at position 44 and circularized via a disulfide bond between the cysteine residues at positions 44 and 57 (C[V44C] 45-57) (Figure 5B), or a cyclic fused peptide of β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus by a peptide bond to the N-terminus of amino acids 45-57 (SEQ ID NO:6) and circularized via a disulfide bond between the cysteine residues at positions 110 and 57. In another preferred

embodiment, the peptide is a cyclic branched peptide of β-hCG amino acids 44-57 (SEQ ID NO:12) with cysteine substituted for valine at position 44 and circularized via a disulfide bond between the cysteine residues at positions 44 and 57 and positions 47 and 51 substituted with a diaminobutyric acid residue on which a proline is peptide bonded to its free amino sidechain.

Cyclization can be, for example, but not by way of limitation, via a disulfide bond between two cysteine residues or via an amide linkage. For example, but not by way of limitation, disulfide bridge formation can be achieved by (1) dissolving the purified peptide at a concentration of between 0.1.-0.5 mg/ml in 0.01 M ammonium acetate, pH 7.5; (2) adding to the dissolved peptide 0.01 M potassium ferricyanide dropwise until the solution appears pale yellow in color and allowing this solution to mix for 24 hours; and (3) concentrating the cyclized peptide to 5-10 ml of solution, repurifying the peptide by reverse phase-high pressure liquid chromatography (RP-HPLC) and finally lyophilizing the peptide. In a specific embodiment, in which the peptide does not contain two appropriately situated cysteine residues, cysteine residues can be introduced at the amino-terminus and/or carboxy-terminus and/or internally such that the peptide to be cyclized contains two cysteine residues spaced such that the residues can form a disulfide bridge. Alternatively, a cyclic peptide formed by an amide linkage can be obtained by, for example but not limited to, the following procedure: An allyl protected amino acid, such as aspartate, glutamate, asparagine or glutamine, can be incorporated into the peptide as the first amino acid, and then the remaining amino acids are coupled on. The allyl protective group can be removed by a two hour mixing of the peptide-resin with a solution of tetrakistriphenylphophine palladium (0) in a solution of chloroform containing 5% acetic acid and 2.5% N-methylmorpholine. The peptide resin can be washed three times with 0.5% N,N-diisopropylethylamine (DIEA) and 0.5% sodium diethyldithiocabamate in DMF. The

amino terminal Fmoc group on the peptide chain can be removed by two incubations for 15 minutes each in 20% piperdine in DMF, and washed three times with DMF for 30 seconds each. The activator mix, N-methylmorpholine and HBTU in DMF, can be brought onto the column and allowed to couple the free amino terminal end to the carboxyl group generated by removal of the allyl group to cyclize the peptide. The peptide can cleaved from the resin as described in the general description of chemical peptide synthesis above and the peptide purified by reverse phase-high pressure liquid chromatography (RP-HPLC). In a specific embodiment, in which the peptide to be cyclized does not contain an allyl protected amino acid, an allyl protected amino acid can be introduced into the sequence of the peptide, at the amino-terminus, carboxy-terminus or internally, such that the peptide can be cyclized.

β-hCG peptides can also be obtained by recombinant expression techniques. (See, *e.g.*, Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, 2d Ed., Cold Spring Harbor, New York, Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K., Vol. I, II). The nucleic acid sequence encoding hCG has been cloned and the sequence determined (see Figure 4 and Xia, H., 1993, *J. Molecular Endocrinology* June 10; 1993:337-343; Sherman, G.B., 1992, *J. Molecular Endocrinology*, June 6, 1992:951-959; Gieseman, L.K. (ed.), 1991, *Basic and Chemical Endocrinology*, pp. 543-567; Ward et al., 1991, in *Reproduction in Domestic Animals*, 4th ed., P.T. Coppos, ed., pp. 25-80, Academic Press, New York) and can be isolated using well-known techniques in the art, such as screening a library, chemical synthesis, or polymerase chain reaction (PCR).

To recombinantly produce β-hCG peptides, nucleic acid sequence encoding the β-hCG peptide is operatively linked to a promoter such that the β-hCG peptide is produced from said sequence. For example, a vector can be introduced into a cell, within which cell the vector or a portion thereof is

expressed, producing the β-hCG peptide. In a preferred embodiment, the nucleic acid is DNA if the source of RNA polymerase is DNA-directed RNA polymerase, but the nucleic acid may also be RNA if the source of polymerase is RNA-directed RNA polymerase or if reverse transcriptase is present in the cell or provided to produce DNA from the RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in bacterial or mammalian cells. Expression of the sequence encoding the β-hCG peptide can be by any promoter known in the art to act in bacterial or mammalian cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, *Nature* 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, *Cell* 22:787-797), the HSV-1 (herpes simplex virus-1) thymidine kinase promoter (Wagner et al., 1981, *Proc. Natl. Acad. Sci. USA* 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, *Nature* 296:39-42), etc., as well as the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, *Cell* 38:639-646; Ornitz et al., 1986, *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409; MacDonald, 1987, *Hepatology* 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, *Nature* 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, *Cell* 38:647-658; Adames et al., 1985, *Nature* 318:533-538; Alexander et al., 1987, *Mol. Cell. Biol.* 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al.,

1986, *Cell* 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, *Genes and Devel.* 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, *Mol. Cell. Biol.* 5:1639-1648; Hammer et al., 1987, *Science* 235:53-58), alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, *Genes and Devel.* 1:161-171), beta-globin gene control region which is active in erythroid cells (Mogram et al., 1985, *Nature* 315:338-340; Kollias et al., 1986, *Cell* 46, 89-94), myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, *Cell* 48:703-712), myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, *Nature* 314:283-286), and gonadotropin releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, *Science* 234:1372-1378). The promoter element which is operatively linked to the nucleic acid encoding the β-hCG peptide can also be a bacteriophage promoter with the source of the bacteriophage RNA polymerase expressed from a gene for the RNA polymerase on a separate plasmid, *e.g.*, under the control of an inducible promoter, for example, a nucleic acid encoding the β-hCG peptide operatively linked to the T7 RNA polymerase promoter with a separate plasmid encoding the T7 RNA polymerase.

In a less preferred embodiment, peptides can be obtained by proteolysis of hCG or β-hCG followed by purification using standard techniques such as chromatography (*e.g.*, HPLC), electrophoresis, etc.

Also included within the scope of the invention are β-hCG peptide derivatives which are differentially modified during or after synthesis, *e.g.*, by benzylation, glycosylation, acetylation, phosphorylation, amidation, pegylation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. In specific embodiments, the peptides are acetylated at the N-terminus and/or amidated at

the C-terminus. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

In another embodiment, the β-hCG peptide derivative is a chimeric, or fusion, protein comprising a functional β-hCG peptide (or two or more portions of β-hCG joined by peptide bond(s)) joined at its amino- or carboxy-terminus via a peptide bond to an amino acid sequence of a different protein. In one embodiment, such a chimeric protein is produced by recombinant expression of a nucleic acid encoding the protein (comprising a β-hCG-coding sequence joined in-frame to a coding sequence for a different protein). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a chimeric product may be made by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer.

6.2.2. FRACTIONS OF SOURCES OF hCG OR β-hCG

In a preferred embodiment, a fraction, particularly a size fraction, of a source of hCG or β-hCG active in treating or preventing wasting syndrome, particularly a size fraction of approximately 40 kD, 15 kD or 2-3 kD, is used to treat or prevent wasting syndrome. The utility of β-hCG peptides and fractions of hCG and β-hCG sources may be determined by the *in vitro* and *in vivo* assays described in Section 6.3 *infra* or by any other method known in the art.

The present inventors have found that different preparations of native hCG and β-hCG have variable effects on HIV infections and Kaposi's Sarcoma both *in vitro* and *in vivo* and on wasting syndromes in animal models. Specifically, the inventors found that among the commercial preparations of (non-recombinant) hCG they investigated, hCG from Fujisawa was the most effective, hCG APL™ (Wyeth-Ayerst) the next most

effective, and PREGNYL™ (Organon) the next most effective in inhibiting HIV infection and replication. A highly purified hCG preparation and recombinant β-hCG were found not to be active against HIV infection and Kaposi's Sarcoma cell growth *in vitro*. In fact, the present inventors have shown that specific size fractions of an active hCG preparation (APL™; Wyeth Ayerst) and human early (*i.e.* first trimester) pregnancy urine have anti-HIV activity, anti-KS activity, and activity against wasting syndrome in transgenic mice, as described *infra* in Section 8. These active fractions were eluted from the sizing matrix as or close to (i.e., within 5 fractions when the fractions are 4 ml fractions eluted from a SUPERDEX™ 200 column (26 mm^2 x 60 mm)) the fractions containing or that would contain material that is approximately 40 kD (±8 kD), 15 kD (±3 kD) and 2-3 kD (±2 kD) molecular weight. One skilled in the art would understand that these fractions could be subjected to further size fractionation to further isolate the component of these fractions having the anti-HIV and/or anti-KS activity and/or anti-wasting activity. Additionally, other methods of fractionation, such as ion-exchange chromatography, affinity chromatography are well known in the art; those skilled in the art would be able to use any available fractionation techniques to obtain the active fractions from the active hCG preparations and human pregnancy (preferably early, *i.e.* first trimester) urine. hCG preparations and fractions of hCG preparations can be screened for efficacy in treating or preventing HIV infection by the assays described in Sections 6.3, 7 and 8 *infra* or by any method known in the art.

In a specific embodiment, the invention provides a first composition comprising one or more first components of a second composition comprising native hCG or native β-hCG, said first components being separated from other components of the hCG sample, said first components being active to inhibit Kaposi's sarcoma, and said second composition being active to inhibit Kaposi's sarcoma, and said native hCG or native β-hCG not being purified to homogeneity in said second

composition. In particular the invention provides a composition comprising components which have been separated from other components of the native hCG or native β-hCG sample by sizing column chromatography, preferably where the components elute from a gel filtration, preferably a SUPERDEX™ 200, sizing column with an apparent approximate molecular weight of 40 kD, 14 kD or 2-3 kD as determined relative to the elution of a native hCG heterodimer, having a molecular weight of 77 kD, and a β-hCG core protein (β-hCG amino acids 6-40 linked via a disulfide bond to β-hCG amino acids 55-92, as depicted in Figure 4 (SEQ ID NO:2)), having a molecular weight of 10 kD.

6.2.3. <u>FRACTIONATION OF SOURCES OF hCG OR β-hCG</u>

The present inventors have found that the component(s) of a source of hCG having anti-HIV and/or anti-KS activity can be further isolated by fractionation of the source of hCG. The inventors have fractionated the active portions of the commercial hCG preparation APL™ (Wyeth-Ayerst) and human early pregnancy urine as described in Section 8 *infra*. Other sources of hCG include but are not limited to urine from women in the second and third trimester of pregnancy, urine from proteinuria patients (both pregnant women with preeclampsia and patients with nephrotic syndromes), urine from patients with hCG secreting tumors, and pituitary glands. However, those skilled in the art will appreciate that any source of hCG or β-hCG having anti-HIV, anti-KS and/or anti-wasting activity can be fractionated to further isolate the active components. The source of hCG of β-hCG can be fractionated using any technique available in the art for the separation and isolation of molecules, for example but not limited to, sizing chromatography, ion-exchange chromatography, affinity chromatography, etc.

Briefly, by way of example but not by way of limitation, urine can be prepared for fractionation as follows:

Urine is collected and stored either frozen or refrigerated for not more than two (2) days. Then,

sodium azide is then added at a concentration of 1 gram/liter and the sample is stored frozen until sufficient material is collected for the fractionation.

At this point, the urine is thawed over night, the pH adjusted to 7.2 to 7.4 with sodium hydroxide and then centrifuged to remove any precipitate (alternatively, the precipitate can be allowed to sediment, *e.g.*, for 1 hour at room temperature, approximately 75% of the supernatant is decanted, the remainder of the supernatant and the precipitate is centrifuged to pellet the precipitate, and the supernatant decanted and added to the first volume of decanted supernatant). The urine is then filtered through, *e.g.*, a 45 micron filter to remove any remaining particulate matter.

Next, the urine is concentrated using any concentration method available in the art which does not remove higher molecular weight material, *e.g.*, material larger than 3,000 daltons in molecular weight; for example, the material may be concentrated using a Pellicon (Millipore) filtration system with a membrane filter cassette having a molecular weight cut off of 3,000 daltons. Concentration with the Pellicon filtration system using the 3,000 molecular weight membrane filter cut off concentrates 30 liters of urine to 500 ml (i.e., a 60-fold concentration) overnight.

To remove salts and lipids, the concentrate can then be passed over a column containing a large volume of Sephadex G25 resin in 0.05 M ammonium bicarbonate (for example, 250 ml of the concentrate can be passed over a column of approximately 1.7 liters, washing the column with 25% ethanol between runs to remove adsorbed lipids and glycoprotein). The resulting desalted and delipidated urine concentrate is then lyophilized.

The lyophilized urine material or commercial hCG preparation (or any source of hCG or β-hCG) is resuspended in either phosphate buffered saline (PBS-- 30 mM sodium phosphate buffer, pH 8.3) or in 0.10 M ammonium bicarbonate

at a concentration and in a volume appropriate for the column upon which the sample will be loaded, for example, but not limited to 0.5 grams of protein in 6 ml (i.e., approximately 83 mg/ml). It is within the skill of the skilled artisan to determine the concentration and volume of the sample to be subjected to fractionation.

The sample can then be fractionated by any method known in the art for the separation of proteins. A preferred method is high resolution gel filtration on a Pharmacia pre-packed SUPERDEX™ 200 column (26/60) by HPLC using any available HPLC apparatus, *e.g.*, with a Hewlett Packard 1050 HPLC equipped with a photodiode array detector. The resuspended sample is loaded onto the column in 30 mM phosphate buffer, pH 8.3, and the material can then be eluted from the column with 30 mM sodium phosphate buffer, pH 7.0; 2M NaCl. Fractionation can also be performed using other types of chromatography matrices e.g., heparin, DEAE-cellulose, Sephadex A50, Sephadex G100, phenyl sepharose, or any sizing, ion-exchange, affinity chromatography or any other chromatography matrix available in the art. The column chromatography can also be run using any method available in the art, e.g., standard gravity flow or low pressure chromatography, FPLC, or reverse phase HPLC.

Many separation techniques are known in the art. Those skilled in the art would understand how to apply these known techniques to the fractionation of hCG preparations.

Many separation techniques are known in the art. Those skilled in the art would understand how to apply these known techniques to the fractionation of hCG preparations.

Once the material has been fractionated, any method known in the art, such as but not limited to, those described in sections 6.3, 7, and 8 *infra*, can be used to determine which fractions have anti-HIV activity and/or anti-KS activity and/or a pro-hematopoietic effect.

When fractionating by size, such as fractionation on the

SUPERDEX™ 200 column, the apparent molecular weight of material in the fractions can be determined by the relative elution of those fractions compared with the elution of specific hCG and β-hCG species having a known molecular weight or with the elution of known protein size markers. In general, proteins elute from a sizing column as a function of their molecular weight. The elution of, for example, hCG and the β-hCG core protein can be determined by assaying the column chromatography fractions for the presence of hCG and the β-hCG core protein, or any hCG or β-hCG species, by any immunoassay technique available in the art, such as radioimmunoassays (either liquid or solid phase), enzyme-linked assays or ELISA assays.

Antibodies, either polyclonal or, preferably, monoclonal, can be generated against hCG or the β-hCG core protein by any method known in the art. Preparation of monoclonal antibodies against hCG and β-hCG species have been described in the art, *see, e.g.*, O'Connor et al., 1994, *Endocrine Reviews* 15:650-683; Krichevsky et al, 1991, *Endocrinology* 128:1255-1264; and Krichevsky et al., 1988, *Endorcrinology* 123:584-593. For the production of antibodies, various host animals can be immunized by injection with hCG, the β-hCG core protein or any other species of hCG, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. For preparation of monoclonal antibodies, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497),

as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Monoclonal cells lines can then be screened for binding to the particular hCG or β-hCG species using the purified species in any type of immunoassay available in the art (*see, e.g.*, Erlich et al., 1985, *Am. J. Reprod Immunol. Microbiol.* 8:48).

The fractions can then be assayed for the presence of the hCG or β-hCG species using a monoclonal antibody specific for the hCG or β-hCG species. The assay can be performed by any method known in the art. For example, an immunoradiometric assay (IRMA) can be used (Krichevsky et al., 1988, *Endocrinology* 123:584-593). Briefly, the IRMA assay is performed by adsorbing an antibody against the hCG or β-hCG species onto the surface of wells of a microtiter plate by incubation in a coating buffer (0.2 M sodium bicarbonate, pH 9.5) overnight at 4°C. The residual non-specific binding sites are blocked by the addition of a 1% bovine serum albumin solution (with 0.1% sodium azide) to the wells for 3 hours at room temperature, and the wells of the microtiter plate are then washed with deionized water. An aliquot of the fraction in assay buffer (0.01 M sodium phosphate, 0.15 M NaCl, 0.01 M EDTA, 0.1% sodium azide, 0.1% bovine γ-globulin, pH 7.4) is incubated in the wells for 24 hours at room temperature. The sample is then removed and the wells washed with deionized water. A solution of a second antibody specific for the hCG or β-hCG species, which antibody has been iodinated with I^{125}, (approximately 40,000 cpm/well) is incubated in the wells for 24 hours at room temperature. The iodinated antibody solution is removed and the wells washed five times with deionized water. The level of radioactivity in each well is then determined in a scintillation counter which can measure γ-irradiation.

6.2.4. GENE THERAPY

In a specific embodiment, nucleic acids comprising a sequence encoding hCG, β-hCG or a β-hCG peptide or a protein derivative thereof, are administered for treatment or prevention of wasting syndrome, by way of gene therapy. Gene therapy refers to therapy performed by the administration of a nucleic acid to a subject. In this embodiment of the invention, the nucleic acid produces its encoded protein that mediates a therapeutic effect by preventing or treating wasting syndrome. For example, any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, *Clinical Pharmacy* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, 1993, *Science* 260:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217; May, 1993, *TIBTECH* 11(5):155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, Current Protocols in Molecular Biology, John Wiley & Sons, NY; and Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY.

In a preferred aspect, a nucleic acid encoding a β-hCG peptide is part of an expression vector that produces the β-hCG peptide in a suitable host. In particular, such a nucleic acid has a promoter operably linked to the nucleic acid sequence coding for the β-hCG peptide, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, a nucleic acid molecule is used in which the β-hCG sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the hCG nucleic acid (Koller and Smithies, 1989, *Proc. Natl. Acad. Sci. USA* 86:8932-8935; Zijlstra et al., 1989, *Nature* 342:435-438).

Delivery of the nucleic acid into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vector, or indirect, in which case, cells are first transformed with the nucleic acid *in vitro*, then administered to the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

In a specific embodiment, the nucleic acid is directly administered *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, *e.g.*, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by infection using a defective or attenuated retroviral or other viral vector (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering it in linkage to a peptide which is known to enter the cell or nucleus, *e.g.*, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see *e.g.*, Wu and Wu, 1987, *J. Biol. Chem.* 262:4429-4432) (which can be used to target cell types specifically expressing the receptors), etc. In a specific embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, *e.g.*, PCT Publications WO92/06180 dated April 16, 1992 (Wu et al.); WO92/22635 dated December 23, 1992 (Wilson et al.); WO92/20316 dated November 26, 1992 (Findeis et al.); WO93/14188 dated July 22, 1993 (Clarke et al.), WO93/20221 dated October 14, 1993 (Young)). In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for

expression, by homologous recombination (Koller and Smithies, 1989, *Proc. Natl. Acad. Sci. USA* 86:8932-8935; Zijlstra et al., 1989, *Nature* 342:435-438).

In a specific embodiment, a viral vector that contains the nucleic acid sequence encoding β-hCG or a β-hCG peptide is used. For example, a retroviral vector can be used (see Miller et al., 1993, *Meth. Enzymol.* 217:581-599). These retroviral vectors have been modified to delete retroviral sequences that are not necessary for packaging of the viral genome. Retroviral vectors are maintained in infected cells by integration into genomic sites upon cell division. The nucleic acid to be used in gene therapy is cloned into the vector, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., 1994, *Biotherapy* 6:291-302, which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., 1994, *J. Clin. Invest.* 93:644-651; Kiem et al., 1994, *Blood* 83:1467-1473; Salmons and Gunzberg, 1993, *Human Gene Therapy* 4:129-141; and Grossman and Wilson, 1993, *Curr. Opin. in Genetics and Devel.* 3:110-114.

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, 1993, *Current Opinion in Genetics and Development* 3:499-503 present a review of adenovirus-based gene therapy. Bout et al., 1994, *Human Gene Therapy* 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be

found in Rosenfeld et al., 1991, *Science* 252:431-434; Rosenfeld et al., 1992, *Cell* 68:143-155; and Mastrangeli et al., 1993, *J. Clin. Invest.* 91:225-234.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., 1993, *Proc. Soc. Exp. Biol. Med.* 204:289-300.) Herpes viruses are other viruses that can also be used.

Another approach to gene therapy, involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including, but not limited to, transfection, electroporation, microinjection, infection with a viral vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see *e.g.*, Loeffler and Behr, 1993, *Meth. Enzymol.* 217:599-618; Cohen et al., 1993, *Meth. Enzymol.* 217:618-644; Cline, 1985, *Pharmac. Ther.* 29:69-92) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. In a preferred embodiment, epithelial cells are injected, *e.g.*,

subcutaneously. In another embodiment, recombinant skin cells (*e.g.*, keratinocytes) may be applied as a skin graft onto the patient. Recombinant blood cells (*e.g.*, hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

In an embodiment in which recombinant cells are used in gene therapy, a nucleic acid sequence coding for hCG, β-hCG or β-hCG peptide is introduced into the cells such that it is expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention.

6.3. <u>DEMONSTRATION OF THERAPEUTIC UTILITY</u>

The invention further provides methods of testing the efficacy of Therapeutics of the invention for treatment or prevention of wasting syndrome. The Therapeutics of the invention are preferably tested in an animal model prior to use in humans.

Specific embodiments provide a method for screening a preparation comprising hCG or an hCG α chain or hCG β chain or a derivative of hCG or of said alpha or beta chain or a fraction of native hCG or native β-hCG, for anti-wasting activity comprising assaying said preparation for the ability to promote weight gain in an animal model that exhibits a wasting syndrome. In one specific embodiment, the hCG preparation is screened by a method comprising measuring the weight of an offspring of an HIV-1 transgenic mouse, which offspring has been exposed to the preparation; and comparing the weight of the offspring which has been exposed to the preparation with the weight of an offspring not so exposed, wherein a greater weight in said exposed offspring indicates

that the preparation has anti-wasting activity. In another specific embodiment, the hCG preparation is screened by a method comprising measuring the change in weight of an SIV infected monkey which has been exposed to the preparation; and comparing the change in weight of the monkey which has been exposed to the preparation to the change in weight of a monkey which has not been so exposed, wherein a greater weight increase or smaller weight loss in said exposed monkey indicates that the preparation has anti-wasting activity.

Any animal model in which wasting occurs can be used. Exemplary tests in animal models are described briefly as follows: First, a Therapeutic of the invention can be assayed in mice transgenic for HIV-1, *e.g.*, mice which have integrated molecular clone pNL4-3 containing 7.4 kb of the HIV-1 proviral genome deleted in the gag and pol genes (Dickie, P., et al., 1991, *Virology* 185:109-119). These mice exhibit cachexia and growth retardation (Franks, R.R., et al., 1995, *Pediatric Res.* 37:56-63). A Therapeutic which reverses the cachexia and growth retardation in the HIV transgenic mice is predicted to have utility for treatment or prevention of wasting syndromes.

Similarly, the efficacy of Therapeutics of the invention can also be assayed in SIV infected rhesus monkeys (see Letrin, N.L., and King, N.W., 1990, *J. AIDS* 3:1023-1040), particularly rhesus monkeys infected with SIV_{mac251}, which SIV strain induces a syndrome in experimentally infected monkeys which is very similar to human AIDS and results in weight loss in the infected monkeys (Kestler, H., et al., 1990, *Science* 248:1109-1112). Specifically, monkeys are infected with cell free SIV_{mac251}, for example, with virus at a titer of $10^{4.5}$ $TCID_{50}$/ml and SIV infection is monitored by the appearance of SIV p27 antigen in PBMCs. An increase in the weight of infected monkeys indicates that the Therapeutic has utility in the treatment of wasting syndrome.

Compounds for use in therapy can be tested in suitable animal model systems prior to testing in humans, including but not limited to rats, mice, chicken, cows, monkeys,

rabbits, etc. For *in vivo* testing, prior to administration to humans, any animal model system known in the art may be used.

Once the Therapeutic has been tested in a non-human animal model, the utility of the Therapeutic can be determined in human subjects. Improvement in wasting syndrome, *i.e.* and increase in body cell mass, can be assessed by any well known clinical techniques available in the art. For example but not limited to, measuring body weight, determination of total body potassium content, determination of intracellular water volume, bioelectrical impedance analysis, anthropometrics and determination of total body nitrogen content (*see, e.g.*, Kotler, D.P. et al., 1985, *Am. J. Clin. Nutr.* 42:1255-65; Ott, M. et al., 1993, *Am. J. Clin. Nutr.* 57:15-19; Miller, T.L. et al., 1993, *Am. J. Clin. Nutr.* 57:588-592). Therapeutics, the administration of which increases body weight or cell mass, should have utility in treatment of wasting syndrome.

6.4. THERAPEUTIC COMPOSITIONS AND METHODS OF ADMINISTRATION

The invention provides methods of treatment and prevention by administration to a subject of an effective amount of a Therapeutic of the invention. The subject is preferably an animal, including, but not limited to, animals such as monkeys, cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human. In a specific embodiment, the subject is a human not afflicted with a cancer which secretes hCG or hCG fragments and, more particularly, not afflicted with Kaposi's Sarcoma.

Various delivery systems are known and can be used to administer a Therapeutic of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the Therapeutic, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a Therapeutic nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to

intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved, for example and not by way of limitation, by topical application, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers.

In another embodiment, the Therapeutic can be delivered in a vesicle, in particular a liposome (*see* Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; *see* generally *ibid.*).

In yet another embodiment, the Therapeutic can be delivered in a controlled release system. In one embodiment, a pump may be used (*see* Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In

another embodiment, polymeric materials can be used (*see* Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); *see also* Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, *i.e.*, the brain, thus requiring only a fraction of the systemic dose (*see, e.g.*, Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

In a specific embodiment where the Therapeutic is a nucleic acid encoding a protein Therapeutic, the nucleic acid can be administered by gene therapy methods as described *supra* Section 6.2.4.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a Therapeutic, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the Therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers,

particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the Therapeutic, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline.

Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The Therapeutics of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the Therapeutic of the invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vivo* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. For example but not by way of limitation, therapy of at least 15,000 I.U. and up to 45,000 I.U. hCG weekly was shown to be effective and well tolerated in humans. Weekly doses of 6,000 I.U. in monkeys and 300-500 I.U. in mice were also shown to be effective. Suitable doses of a β-hCG peptide predicted to be effective for treatment of wasting syndrome in a human include, but are not limited to, 1 to 1000 micrograms of peptide per week, administered, for example but not limited to, intramuscularly, subcutaneously or intravenously. Effective doses may be extrapolated from dose-response curves derived from or animal model test systems.

Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

7. EXAMPLE: EFFECTS OF hCG, β-hCG, AND β-hCG PEPTIDE PREPARATIONS ON WASTING

Human Chorionic Gonadotropin (hCG), a glycoprotein hormone produced in early pregnancy, consists of two sub-units, α and β, which associate noncovalently to form a heterodimer which embodies its hormonal activity. Here we report that some partially purified preparations of hCG, β-hCG, and β-hCG peptides prevent wasting associated with HIV infection. Specifically, in the presence of some preparations of hCG, β-hCG, or β-hCG peptide, the fatal wasting of homozygous transgenic mouse offspring is reversed. Furthermore, in 3 of 3 SIV acutely infected rhesus macaque monkeys the same preparation of hCG (pre-screened for anti viral activity) at a dose of 6,000 IU per week, led to a reduction of SIV in plasma, an increase in $CD4^+$ T-cells and weight gain. Finally, non-fluid weight gain was observed in preliminary studies of a limited number of patients treated with some commercial hCG products. Factors such as patient stage, total weekly dose, and manufacturer source very likely play a role in the variability of response.

7.1. EFFECTS OF hCG PREPARATIONS ON HIV-1 TRANSGENIC MICE

The HIV-1 transgenic mice used for this study contain 7.4 kb of foreign DNA, including 5.1 kb of the HIV-1 proviral genome deleted in the *gag* and *pol* genes and 2.3 kb of vector

(Dickie et al., 1991, *Virology* 185:109-119). The birth weights of mice homozygous for the HIV-1 transgene are normal, but soon the mice uniformly display severe growth retardation (Figure 1A), cachexia, and early mortality from expression of HIV-1 genes (env and regulatory genes) which are highly expressed shortly after birth in homozygotes (Franks et al., 1995, *Pediatric Res.* 37:56-63) (Figure 1B). In addition, these mice develop severe hyperkeratotic skin lesions with marked expression of gp120 and nef genes (Kopp et al., 1993, *AIDS Res. Hum. Retroviruses* 9:267-275; Vasli et al., 1994, *AIDS Res. Hum. Retroviruses* 10:1241-1250).

To examine the effects of hCG preparations on HIV transgenic Tg26 mice, the mothers of 30 neonatal mice were administered a commercial preparation of native hCG (APL™, Wyeth Ayerst) (300-500 I.U.), and the mothers of other HIV-1 transgenic mice received other commercial native hCG preparations (PREGNYL™ and Sigma (G10), α-chain preparations and partially purified native β-hCG and recombinant β-hCG preparations (all Sigma) (50-100 μg). For studies involving synthetic peptides, heterozygous transgenic mothers of 6 homozygous transgenic mice were given 10 μg of β-hCG peptide 45-57 (SEQ ID NO:6) where the amino acid residues at positions 47 and 51 are substituted by a branch, where the branches are made up of diaminobutyric acid and proline (branched β-hCG 45-57) (prepared by Dr. N. Ambulos, UMAB) subcutaneously, daily for 10 days. Other studies were carried out with other β-hCG peptides (see Table 1).

Heterozygous transgenic mothers were treated with the hCG preparation subcutaneously twice weekly. Pups received hCG via the mother's milk. Blood levels of hCG in the mother and pups were monitored and ranged from 5 IU/ml to over 150 IU/ml over a 96 hour time period (data not shown).

Gene expression was assayed in total RNA extracted from the skin of Tg26 mice with RNAzol. One microgram of RNA was reverse transcribed into cDNA using random hexamer primers and MMTV reverse transcriptase (Life Technologies, MD) in a final volume of 30 μl. One tenth of the cDNA reactions were

used for PCR amplification of various HIV gene sequences (env, tat, rev, nef and vif). In addition, glyceraldehyde 3-phosphate dehydrogenase (GAPHD) mRNA was amplified for each sample for normalization. Following 25 cycles of amplification, 10% of the PCR product was resolved by electrophoresis through 2% agarose gels and processed for Southern hybridization using FITC-labeled oligonucleotide probes complementary to internal sequences of the amplicons. Detection was performed by chemiluminescence (Amersham) and relative mRNA levels determined by densitometry after normalization with GAPDH mRNA levels.

The hCG (APL™) treatments resulted in marked down regulation of HIV-1 gene expression in skin biopsies as determined by the RT-PCR technique (Figure 1D). The 25 cycles of amplification employed in these experiments readily detected abundantly expressed genes (*e.g.*, env and rev) while the tat gene in treated animals was at low levels, and more readily detected with more cycles of amplification (not shown). Other skin biopsies were examined for HIV viral proteins using mouse monoclonal antibodies against gp120 and Nef by an immunostaining technique. A marked decrease in viral proteins occurred after 2 weeks treatment and no detectable HIV proteins were found after 30 days of hCG treatment (not shown) and the hyperkeratosis of the skin regressed. When the treatment was halted, reappearance of viral protein expression occurred after 2 weeks (not shown).

Associated with the decrease in HIV-1 gene expression (Figure 1D) was a reversal of the growth retardation and cachexia (Figure 1C). As described immediately below, native β-hCG and some synthetic peptides of the β-subunit also reversed the adverse effects of the viral genes in these transgenic animals (Table 1). In contrast, native α-hCG had no effect on HIV gene expression or the retarded postnatal growth and cachexia (Table 1).

Table 1 and Figures 1A and B also provides results from the administration of various β-hCG peptides and β-hCG peptide derivatives, *i.e.* the peptides of amino acids 45-57

(SEQ ID NO:6) ("Satellin A1"), circularized 44-57 with cysteine substituted at position 44 (SEQ ID NO:26) ("Satellin A2"), 47-57 linked at the C-terminus via a peptide bond to the N-terminus of 108-119 (SEQ ID NO:32) ("Satellin A1/B^G"), 45-57 linked at the C-terminus via a peptide bond to the N-terminus of 109-119 (SEQ ID NO:30)("Satellin A1/B"), 41-54, 6-16, 47-55 (SEQ ID NO:20), and 48-56 (SEQ ID NO:35). All animals born to HIV-1 transgenic mothers which did not receive preparations containing β-hCG peptide or derivative thereof died within 10 days, showed high level of gp120 and nef protein as measured by antibody staining, and exhibited characteristic hyperkeratosis. The pups receiving the β-hCG peptides 45-57, circularized 44-57 with cysteine substituted at position 44, 47-57 linked by peptide bond to 108-119, 45-57 linked by peptide bond to 109-119 (SEQ ID NOS:6, 26, 32 and 30, respectively) all exhibited considerable inhibition of HIV-1 transcription and protein expression and higher weight gain than untreated mice. The peptides 47-55 and 48-56 (SEQ ID NOS:20 and 32, respectively) also elicited inhibition. The scrambled peptide 45-57::109-119 and the scrambled circularized 44-57, administered as controls, showed no inhibitory effect while, surprisingly, the scrambled 45-57 peptide did exhibit some inhibition (Table 1).

Table 1 ACTIVITIES OF hCG AND hCG SUBUNIT PREPARATIONS AND hCG PEPTIDES.

Sources	HIV in vitro	HIV transgenic mice	KS in vitro	KS in vivo	Pro-hematopoiesis in vitro
	Inhibition				Enhancement
hCG preparations					
APL™	+++	+++	+++	+++	+++
PREGNYL™	++	++	++	++	++
ORGANON	-	ND	-	-	ND
PROFASI™	+	ND	+	+	+
GOLDLINE	+	ND	-	-	-
STERIS™	++	ND	++	ND	++
SHEIN	+	ND	-	-	-
SIGMA	+++	+++	+++	+++	+++
SIGMA[2]	-	ND	-	-	-
CR127	-	ND	-	ND	-
CR1XY17V	-	ND	-	ND	-
CR1XY17B	-	ND	-	-	-
rhCG	-	-	-	-	-
hCG subunits					
α Chain					
αhCG	-	-	-	-	-

Sources	HIV in vitro	HIV transgenic mice	KS in vitro	KS in vivo	Pro-hematopoiesis in vitro
			Inhibition		Enhancement
rαhCG	-	-	-	-	-
αfp1769A	-	ND	-	ND	-
β Chain					
rβhCG	-	-	-	-	-
βhCG	++	++	++	++	++
Synthetic peptides β-chain hCG					
1. 109-119	+	ND	+	+	+
2. 109-145	+	ND	+	+	+
3. 45-57	++	++	++	++	++
4. Circ 44-57	+++	+++	+++	+++	+++
5. 47-57::108-119	++	++	++	+++	ND
6. 45-57::109-119	++	++	++	++	++
7. 45-57+109-119	++	ND	++	ND	++
8. 41-54	-	-	-	-	-
9. 38-57	-	ND	-	-	-
10. Scrambled 45-57::109-119	-	-	-	-	-
11. Scrambled 45-57	++	ND	++	ND	ND

Sources	HIV in vitro	HIV transgenic mice	KS in vitro	KS in vivo	Pro-hematopoiesis in vitro
	Inhibition				Enhancement
12. Scrambled circ. 44-57	-	ND	-	ND	-
13. 6-16	-	-	-	-	-
14. 1-20	-	ND	-	ND	ND
15. 20-47	—	ND	—	ND	—
16. 31-50	-	ND	-	ND	-
17. 46-65	+	ND	+	ND	ND
18. 91-112	ND	ND	-	ND	-
19. 93-100	-	ND	-	ND	ND
20. 110-145	ND	ND	-	ND	-
21. 74-95	-	ND	-	ND	-
22. 7-40	+	ND	+	ND	-
23. 57-93	-	ND	-	ND	-
24. 34-39	-	ND	-	ND	ND
25. 123-145	-	ND	-	ND	ND
26. 134-144	-	ND	-	ND	-
27. 100-110	-	ND	-	ND	ND
28. 113-132	ND	ND	-	ND	-
29. 128-145	-	ND	-	ND	-V

Sources	HIV in vitro	HIV transgenic mice	KS in vitro	KS in vivo	Pro-hematopoiesis in vitro
	Inhibition				Enhancement
30. 37-55	+	+	+	+	+
31. 51-59	-	ND	-	ND	-
32. 48-56	+	+	+	+	+
33. Trimers	-	ND	-	ND	-
<u>Synthetic peptides α-chain hCG</u>					
34. 88-92	-	ND	-	ND	ND
35. 1-15	—	ND	-	-	-
36. 16-30	-	ND	-	ND	-
37. 26-45	-	ND	-	ND	-
38. 41-61	-	ND	-	ND	ND
39. 57-76	-	ND	-	ND	ND
40. 72-92	-	ND	-	ND	-
41. 1-95	-	-	-	-	-

In Table 1, "-" represents less than 10% effect; "+" represents greater than 15% effect; "++" represents greater than 40% effect; "+++" represents greater than 70% effect; and "ND" represents no data available. The "HIV: in vitro" column reports results from assays of the inhibition of HIV-1 replication in vitro (HIV-1 strains and HIV-1 primary isolates) as described infra sections 7.4 and 7.6. The "HIV transgenic mice" column reports data from the inhibition of HIV RNA and protein expression in HIV-1 transgenic mice as described in section 7.1. Columns labeled "KS:in vitro" and "KS:in vivo" report on the inhibition of Kaposi's Sarcoma cell growth in vitro in cultured cells and of Kaposi's Sarcoma induced in mice, respectively, as described in section 7.7. Column 5 provides data on the relative increase of hematopoietic colony cell number in vitro clonogenic assays as described in section 7.8. The commercial hCG preparations tested were APL™ (Wyeth Ayerst), PREGNYL™ (Organon), ORGANON (a highly purified preparation obtained from Organon), PROFASI™ (Serono), Goldline, STERIS™, and Shein, and two preparations from Sigma, Sigma[1] (GHO) and Sigma[2] (C1063). The hCG preparations CR127 and CR1XY17V are highly purified hCG preparations and CR1XY17B is a mixture of highly purified α-hCG and β-hCG, all three preparations were obtained from the National Institute of Child Health and Human Development (NICHD) at the National Institute of Health (NIH) and the rhCG is recombinant hCG expressed in a mouse cell line (Sigma). For the hCG subunits "αhCG" and "βhCG" are purified native subunits (Sigma); "rαhCG" and "rβhCG" all the recombinant subunits expressed in mouse cells (Sigma); and αfp1769A is purified, native α subunit (NICHD, NIH). The peptide "scrambled A1" has the sequence Cys-Val-Ala-Gln-Pro-Gly-Pro-Gln-Val-Leu-Leu-Val-Leu-Cys (SEQ ID NO:36) and "Scrambled A2" has the sequence Cys-Val-Ala-Gln-Gly-Val-Leu-Pro-Ala-Leu-Pro-Gln-Val-Val-Cys (SEQ ID NO:37). "Scrambled A1/B" has the sequence β-hCG amino acids 45-57 (SEQ ID NO:6) and 109-119 (SEQ ID NO:7) which has been scrambled. "Trimers" is a mixture of tripeptides from the β-hCG sequence

of amino acids 45-57: Leu-Gln-Gly, Leu-Gln-Pro, Gln-Gly-Val, Gln-Pro-Val, Gln-Val-Leu, Val-Leu-Pro, Leu-Pro-Ala, Leu-Pro-Pro, Pro-Ala-Leu, Pro-Pro-Leu, Ala-Leu-Pro, Pro-Leu-Pro, Leu-Pro-Gln, Pro-Gln-Val, Gln-Val-Val, and Val-Val-Cys (SEQ ID NOS: 38-53, respectively). Peptides were synthesized by Dr. N. Ambulos (University of Maryland Biomedicine Center), Becham (CA) or Peptide Technologies Corp. (Gaithersburg, MD).

7.2. EFFECTS OF hCG PREPARATIONS IN SIV INFECTED RHESUS MONKEYS

Events early in HIV infection are thought to be critical to subsequent AIDS pathogenesis. Although the early events in HIV infection are difficult to study in humans, they can be readily investigated in the SIV infected rhesus monkey animal model (Letvin et al., 1990, *J. AIDS* 3:1023-1040). SIV and HIV-1 are similar in many of their biological and physical properties including their genomic structure. In addition, SIV_{mac251}, unlike several other SIV isolates, induces a syndrome in experimentally infected rhesus macaques that is similar to human AIDS (Kestler et al., 1990, *Science* 248:1109-1112).

The effect of the same commercially available hCG preparation (APL™, Wyeth Ayerst), which had been prescreened for anti-viral and anti-KS activity, was studied in five adult male rhesus monkeys who were intravenously inoculated with cell free SIV_{mac251} ($10^{4.5}$ $TCID_{50}$/ml). In all animals, SIV p27 was apparent in plasma 14 days after infection, reaching a maximum by about day 20 (not shown). Treatment with systemic injections (3,000 IU, 2 times weekly) of the active commercial preparation of hCG (APL™), was initiated 3 weeks after SIV inoculation. Two months post-inoculation, the characteristic increase of SIV p27 antigen (Figure 2A), reduction of $CD4^+$ T cells (Figure 2B), and weight loss (Figure 2C) occurred in 2 of 2 untreated infected monkeys. In contrast, the 3 infected monkeys treated with this hCG preparation showed weight gain characteristic of uninfected animals of this age (Figure 2C), a marked decrease in SIV p27

(Figure 2A) and an increase in CD4+ T cells to normal levels (Figure 2B). These effects were maintained over the 7 months the animals were followed. These results show that this commercially available hCG preparation can control SIV_{mac251} acute infection, increase CD4+ T cells, and promote weight gain in SIV infected rhesus monkeys and that these effects can be maintained. The animals were followed for 7 months, and no evidence of disease or SIV resistance to the hCG preparation developed.

In Figure 2D, results are shown from 4 uninfected controls: 2 received the hCG preparation and 2 received the diluent without the hCG. There is a slight increase in the CD4+ T cells in the treated animals (increasing from 460 mm^3 to 520 mm^3 and from 470 mm^3 to 650 mm^3) (Figure 2D). The 2 treated animals also showed a 1 to 2 kg weight gain (not shown).

7.3. EARLY STUDIES OF SOME hCG PREPARATIONS IN PATIENTS WITH HIV-1 DISEASE

The incidence of KS is greatly increased in HIV-infected persons (Friedman-Kien et al., 1981, *J. Am. Acad. Dermatol.* 5:468-473). Based on experimental studies of the killing effect of some hCG preparations on KS Y-1 cells, clinical trials with some commercially available preparations of hCG given either intralesionally (Hermans et al., 1995, *Cellular and Molecular Biology* 3:357-364; Gill et al., 1996, *New Engl. J. Med.* 335:1261-1269, Harris, P.J., 1995, *The Lancet* 346:118-119) or systemically to KS patients have shown that cutaneous KS lesions were reduced via cell killing by apoptosis following intralesional inoculation (Lunardi-Iskandar et al., 1995, *Nature* 375:64-68; Hermans et al., 1995, *Cellular and Molecular Biology* 3:357-364; Gill et al., 1996, *New Engl. J. Med* 335: 1261-1269) and induced regression of advanced KS disease treated by systemic delivery.

Clinical trials reported herein were undertaken in Belgium and California to evaluate the anti-KS properties of

systemic hCG therapy with or with out concomitant intralesional therapy. Use of anti-viral protease and non-protease inhibitors was not restricted. A total of 47 patients were enrolled under protocols of compassionate use sanctioned by the Institutional Review Boards of the respective centers. 29 patients were treated in Belgium, either on a protocol to investigate intralesional and systemic treatment of cutaneous KS (n=15), or in the pre-clinical phase of that protocol (n=4), or on compassionate use for systemic KS or HIV infection (n=10). The protocol involved intralesional administration of 500 IU hCG (PREGNYL™) to 4 lesions for 2 weeks, followed by subcutaneous administration of 2,500 IU hCG (PREGNYL™) 5 days per week for 4 to 6 weeks. Additional systemic intramuscular or subcutaneous hCG treatment with either PREGNYL™, APL™, or STERIS™ (one patient) was provided as ongoing therapy in some patients or as part of compassionate use protocols.

A total of 18 patients were treated in California with at least 1 month of follow-up as part of an ongoing protocol to evaluate systemic hCG therapy for cutaneous KS. These patients received either 5000 IU of APL™ subcutaneously 7 days per week, 10,000 IU subcutaneously 3 times per week, or 10,000 IU subcutaneously 7 days per week.

Overall 30 patients were on pre-existing, anti-viral therapy (19 on RT inhibitors and 11 on protease inhibitors), 11 were on no anti-virals and 8 were missing information. One patient, PH-RF, was on 3TC therapy before hCG therapy, and despite poor compliance, had an hCG response for visceral KS and viral load, which declined to undetectable on hCG alone.

Thirty-six patients survived the study, 7 (PH-LFA, PH-DD, PH-PJ, PO-BO, PO-RB, PH-JJ, PH-MH) died either from opportunistic infections or multiple organ failure. The vital status of 1 patient is unknown. Two patients, PH-DD and PH-OJ, discontinued hCG treatment because of cholestasis. PH-DD was on concomitant anti-mycobacterial therapy which was felt to be a contributing factor. PH-OJ had preexisting

cholestasis, which was exacerbated by the hCG treatment with a marked increase in alkaline phosphatase and rise in bilirubin which required hospitalization (PH). These values declined by 2-fold following discontinuation of hCG therapy. These cases raise the possibility that liver toxicity may be a rare complication of hCG therapy.

Early clinical experience with relatively low dose intralesional hCG administration for KS indicated partial or complete regression of treated lesions, including 3 of the first 4 patients in the initial pilot study in Belgium (Hermans et al.,1995, *Cellular and Molecular Biology* 3:357-364) as well as a dose dependent effect between 16% (250 IU) and 83% (2,000 IU) in patients reported from California (Gill et al., 1996, *New Engl. J. Med.* 335:1261-1269), and other cases showing striking clearance of visceral (lung and gastrointestinal) KS in very advanced disease following systemic therapy with hCG APL™ or PREGNYL™ within 1 to 3 months of initiating therapy.

Among the 30 cases with cutaneous Kaposi's Sarcoma, 12 were treated with intralesional followed by systemic therapy in Belgium and 18 with systemic therapy only in California. Complete (2/12, Belgium; 2/18, California) and partial (5/12, Belgium; 4/18, California) responses were observed while progressive disease was noted among 2/12 from Belgium and 10/18 in California. The overall response rate for the study (CR + PR) was 43% (13/30). The response rate in the group administered hCG both intralesionally plus systemicly group was 58%, while the response rate was 33% in the group receiving only the systemic treatment. Among 8 patients with both visceral and cutaneous KS treated in Belgium with very advanced pulmonary or gastric lesions, 3 patients experienced complete remissions, 2 patients exhibited tumor stabilization and 3 progressed, in each case after failure of conventional cytotoxic therapy.

AIDS patients treated with hCG therapy were tested for increases in $CD4^+$ T cell levels (in numbers of cells per mm^3) and decrease in viral load by one of the following assays for

determining viral load: NASBA (Louache, et al., 1992, *Blood* 180:2991-2999; Geller, et al., 1985, *Archs. Path. Lab. Met.* 109:138-145), which has a lower detection limit of 4,000 copies; Roche Amplicor, with a lower detection limit of 200 copies; RT-PCR, with a lower detection limit of 100 copies; or TCID assay in which the infection of PBMCs in co-culture is determined (Popovic et al., 1984, *Science* 204:497-500). As viral load was assayed retrospectively, the viral load results were not a factor in guiding choice of therapy or changes in therapy. Each patient served as their own control and change in viral load (0.7 log change between baseline and subsequent post hCG viral load, scored as significant) was the endpoint measurement for this analysis. For analysis of the anti-viral effect, in addition to the 10 patients undergoing with synchronous hCG and other anti-viral therapy, 6 patients were excluded because of a lack of base line viral load or insufficient follow up before hCG therapy was stopped or additional anti-viral therapy was started.

Among the 16 cases, 1 (PH-OJ) experienced a fall in viral load of 0.7 log on 2 successive tests at least 1 month apart while on stable anti-viral therapy (see Figures 3A and B), 11 were non responding and 2 (PH-VE and PHGRX) manifested an increase in viral load of at least 0.7 log after hCG therapy on 2 successive tests at least 1 month apart. As illustrated in Figures 3C and D, another patient (PG-1), initially on hCG alone and classified as non responsive by study criteria (2 consecutive values of 0.7 log decrease in viral load over 1 month) on hCG alone, experienced a steady decline in viral load but the second qualifying >0.7 log viral load drop was measured 2 weeks after non protease inhibitor therapy was begun. Because of this short window, it is likely that this second stable viral load point is accounted for by hCG rather than the newly introduced anti-virals. It is noteworthy that CD4^{+} T cell levels were not significantly altered in this case but, the patient's KS progressed, documenting a dissociation of various hCG effects.

Among the 6 cases being treated with hCG alone (i.e. without other anti-viral therapies) with analyzable data, all were scored as non responsive to the hCG therapy by the scoring criteria although one case (PG-1) noted above (and illustrated in Figures 3C and D) is a probable responder. An additional patient on hCG alone (PG-8; Figures 3E and F) experienced a sustained fall in viral load of 0.5 log over a 2.8 month period of treatment on hCG alone until KS lesions progressed, at which time hCG therapy was discontinued. Thus of the 7 analyzable patients on hCG alone, 4 exhibited a downward trend in viral load, 2 patients showed an increase in viral load, and 1 patient was stable.

To more fully evaluate all data from patients on hCG alone or with stable antiviral therapy, all eligible data points were plotted, as shown in Figure 7A, indicating the coordinates for each data point pre and post therapy, with values on the line representing no change in viral load. Values are distributed more or less equally above and below the line with no obvious trend to suggest a strong anti viral effect. To evaluate a dose response relationship between hCG and viral load, regression analysis for patients on hCG, alone or with stable antiviral therapy is shown in Figure 7C. There was no detectable effect of higher hCG dose on viral load level ($r=-.089$, $p=0.285$, $N=147$ measurements). An analysis by different CD4 strata did not show any significant trends to suggest that level of immunity impacted the hCG effect.

Among the 22 patients with analyzable $CD4^+$ T cell data, 5 demonstrated a pro-$CD4^+$ T cell effect (PH-VE, PH-RF, PG-9, PG-17, and PG-19) characterized by a 50% rise in $CD4^+$ T cell count sustained over at least a one month period, as demonstrated by plotting the data from at least two patients (PH-VE--Figures 3G and H and PG-17--Figures 3I and J). Of these 5 patients, concomitant stable non protease anti-virals were administered to 2 patients, stable protease inhibitors in 2 cases and hCG preparation alone in 1 case. Thus of the 6 cases with valid $CD4^+$ T cell data on hCG preparation alone,

1 manifested a significant response. No patient experienced an adverse fall in $CD4^+$ T cell on hCG preparation therapy, although patient PH-VE experienced an 0.7 log rise in viral load with a sustained 50% fall in $CD4^+$ T cell numbers and a partial anti KS response (Figures 3G and H). Similarly, paient PG-17 experienced a significant rise in $CD4^+$ T cells and no change in viral load on hCG therapy alone, yet experienced progression of KS after 2.5 months (Figures 3I and J). All $CD4^+$ T cell values (except for 2 patients on hCG alone) were at or above baseline, with the most significant rises in those on concomitant stable protease inhibitor or non protease drugs (Figure 7B). There is no correlation between a change in the $CD4^+$ T cells count and the dosage of hCG (r=.101, p=.339, N=92) (data not shown).

Among the 26 patients analyzable for weight gain (patients who started hCG preparation therapy coincident with or shortly after starting other anti-viral therapy were excluded), 14 gained weight, 3 experienced weight loss, and 9 remained stable. There was no correlation between weight change and dosage of hCG (data not shown). There was however a pattern observed in some patients where an initial weight gain was followed by a return to baseline levels while others experienced sustained weight gain over several months.

hCG therapy was well tolerated clinically by patients and there was no evidence for an adverse effect of hCG on viral load or $CD4^+$ T cell level. In two cases with advanced HIV disease hCG was discontinued because of coincident cholestasis probably due to other medications in one case and opportunistic infections in the other.

7.4. DISCUSSION

Certain preparations of hCG and β-hCG were particularly efficacious in reversing wasting associated with HIV or SIV infection although variability among different preparations was observed. The native hCG and native β-chain preparations available for clinical use are not homogenous and may be contaminated with one or more other active molecules. In

this respect, it is noteworthy that though the effects of some preparations of hCG described here were obtained with two different commercial sources of hCG (APL and Pregnyl), one was usually more active (APL) at lower concentrations than any other preparation despite the fact that identical amounts (International Units) were used, as assessed by the manufacturer's standard bioassays for the conventional use of hCG. The differences in activities of commercial preparations might be explained by variation in the amount of β-hCG peptide fragments. This could be the consequence of different methods of preparation or different sources of human urine. For example, free β chain is more abundant in the earliest weeks of pregnancy.

8. EXAMPLE: FRACTIONATION OF ACTIVE hCG PREPARATIONS AND HUMAN EARLY PREGNANCY URINE

The present inventors have found that certain commercial preparations of hCG, for example, hCG APL™ (Wyeth-Ayerst), had higher anti-HIV, anti-Kaposi's Sarcoma, anti-wasting and pro-hematopoietic activity than other commercial preparations of hCG (see discussion in section 7 *supra*). Further, the inventors have also shown that highly purified preparations of native and recombinant hCG and β-hCG had no activity against HIV infection or replication or against Kaposi's Sarcoma (see results discussion section 7 *supra*). Accordingly, the inventors postulated that there must be an activity in the hCG commercial preparations that is not the hCG dimer or the β-hCG subunit, responsible for the anti-HIV, anti-Kaposi's Sarcoma, anti-wasting and pro-hematopoietic activities. This section presents results of the fractionation of the APL™ hCG commercial preparation and urine from women in the first trimester of pregnancy ("human early pregnancy urine") which also contains hCG. Particular sizing column chromatography fractions were shown to have activity, thus demonstrating that the active components could be fractionated.

8.1. MATERIALS AND METHODS

Both human early pregnancy urine and the APL™ (Wyeth-Ayerst) hCG commercial preparation were subjected to fractionation. For the human early pregnancy urine, 5 liters of urine were collected from women in the first trimester of pregnancy. Twenty-four hour collections were stored frozen or refrigerated for up to 2 days. Upon delivery of the urine to the laboratory, sodium azide was added at 1 g/liter and the urine frozen until five liters had been collected. At this time, all the urine was thawed overnight, and the pH was adjusted to 7.2-7.4 with NaOH, which causes some precipitation. The precipitate was allowed to sediment for 1 hour at room temperature, most of the supernatant decanted and the remaining supernatant centrifuged to remove any additional precipitate with that supernatant being added to the first supernatant decanted. Next, the urine was concentrated with a Pellicon (Millipore) filtration system using a membrane cassette with a 3,000 MW cut off, which concentrates the urine approximately 60 to 80 fold. Next, the urine was desalted and delipidated by passing 500 ml of the material at a time through a Sephadex G25 column with a volume of 1.7 liters in 0.05 M ammonium bicarbonate (the column was washed between runs with 25% ethanol to remove absorbed lipids and glycoprotein). The material was lyophilized and stored for further fractionation. The urinary material was then reconstituted in 6 ml of 30 mM sodium phosphate buffer, pH 8.3 fractionation.

For the APL™ hCG, the lyophilized hCG preparation from eleven vials (each vial containing 20,000 IU hCG) was resuspended in 6 ml of 30 mM sodium phosphate buffer, pH 8.3 and filtered twice through the 0.45 μm particle filter. For both the lyophilized urine and the hCG APL™, the prepared sample was then loaded onto a pre-packed SUPERDEX™ 200 HiLoad Column (Pharmacia 26 mm^2 x 60 cm) in the 30 mM sodium phosphate buffer, pH 8.3 and then eluted from the column with a solution containing 30 mM sodium phosphate buffer, pH 7.0 and 2 M NaCl. For the first ten minutes, the column flow

rate was 1 ml/minute (due to the viscosity of the hCG APL™ material; this flow rate scheme was also used for the urine material); after the first 10 minutes, the flow rate was 2 ml/minute. The column was run on a Hewlett Packard 1050 HPLC equipped with a photodiode array detector. Four ml fractions were collected and frozen until further analysis.

The protein concentration in each fraction was determined by amino acid analysis. A 50 μl aliquot of alternate column fractions was processed for anlysis by hydrolysis in vapors of 6N HCl with 0.1% phenol at 110°C for 24 hours in a Waters Associates Pico-Tag Workstation (Waters, Milford, MA). An internal standard, norleucine, was added to all fraction samples before hydrolysis to correct for any losses during hydrolysis or liquid transfer. The hydrolyzed samples were then analyzed on a Beckman Instruments 6300 amino acid analyzer and the data was collected on the PE Nelson Data System (Perkin-Elmer) and transformed using PE Nelson Turbochrome software.

The column fractions were monitored with immunoassays to heterodimeric hCG as well as to the hCG beta core fragment (O'Connor et al., 1994, *Endocrin. Rev* 15:650-683; Krichevsky et al., 1994, *Endocrinology* 134:1139-145; Krichevsky et al., 1991, *Endocrinology* 128:1255-1264; O'Connor et al., 1988, *Cancer Res.* 48:1361-1366; Krichevsky, 1988, *Endocrinology* 128:584-593). These two assays permit placement of two internal standard sizes for the gel filtration column: 70,000 kD (hCG) and 10,000 kD (hCG beta core fragment which is amino acids 6-40 of β-hCG linked via a disulfide bond to amino acids 55-92 of β-hCG). External molecular weight standards were also employed to calibrate the column elution positions. In addition, MALDI-TOF mass spectrometry was used to evaluate the ions observed in certain active fractions. Mass spectrometry did indicate that some peptides separated at anomalous positions, showing that they were being carried by other proteins to earlier elution positions in some cases, or interacting with the column matrix and eluting much later than their molecular size would indicate. For example, 3,000

and 6,000 molecular weight materials eluted from the gel filtration column with material of 14,000 molecular weight while 11,000 molecular weight material eluted with material of approximately 1,000-2,000 molecular weight, hCG and hCG-related molecules eluted at their expected positions.

The fractions were then tested for anti-HIV, anti-KS and pro-hematopoietic activities in vitro. To assay for inhibition of HIV-1 replication in vitro, the HIV-1 IIID viral strain was used to infect PBMCs and PM-1 cells (derived from the HUT-78 T-cell lymphoma cell line) at 10^3 $TCID_{50}$/ml. The infected cells were incubated for three days in 100 IU/ml of the hCG APL™ or β-hCG C-Sigma preparations; 50-100 μl per ml of the hCG APL™ or early pregnancy urine fractions; 50 μg/ml β-core protein or α-hCG preparation; 200 IU/ml of the highly purified CR127 hCG preparation; or 100 μl/ml of the circularized β-hCG peptide 44-57 (with cysteine substituted at position 44; SEQ ID NO:26). Viral levels were determined by measurement of p24 antigen.

To assay for activity against Kaposi's sarcoma cell growth in vitro, clonogenic assays were performed with the KS Y-1 and KS-SLK cultured Kaposi's Sarcoma cell lines (Lunardi-Iskandar et al., 1995, *Nature* 375:64-68; Nakamura et al., 1988, *Science* 242:426-430; Ensoli et al., 1989, *Science* 243:223-226; Salahuddin et al., 1988, *Science* 242:430-433; Masood, et al., 1984, *AIDS Res. Hum. Retroviruses* 10:969-976). Briefly, the KS Y-1 cells were obtained from mononuclear cells isolated from pleural effusion of an AIDS patient with KS in the lungs. After the depletion of T lymphocytes, monocytes/macrophages and fibroblasts using monoclonal antibodies against CD2, CD3, CD4, CD8, CD10 and CD14 membrane antigens and baby rabbit complement, the cells were cultured in the absence of exogenous growth factors to select for transformed cells. Immunological characterization of the KS Y-1 cells showed that CD34, CD31 and endoglin were expressed. Clonogenic assays were performed by seeding the KS Y-1 or KS-SLK cells in methylcellulose (0.8%, v/v), incubating the cells for 10 days in the presence or absence

of the test substance and then counting the number of well-formed colonies of triplicate wells formed after seeding with 5 X 10^4 cells. The cells were incubated in 200 IU/ml of commercial hCG preparations; 50 μl/ml of certain fractions from the hCG preparation of early pregnancy urine fractionation; or 100 μg/ml β- and α-hCG chains, β-hCG core protein, β-hCG peptides or LH (leuteinizing hormone).

Finally, certain fractions were tested for their ability to increase survival, promote weight gain and reduce HIV-1 gene expression in HIV-1 transgenic mice as described in Section 7.1 *supra*. The mothers the mice were administered 300 IU hCG APL™ by osmotic pump or 200 IU hCG APL™ by slow release; 200 μl of certain fractions of hCG commercial preparation or of early pregnancy urine; 200 μg of the cyclized β-hCG peptide of amino acids 44-57 (with cysteine substituted at position 44; SEQ ID NO:26) or the fused peptide of amino acids 45-57::109-119 (SEQ ID NO:30); or 100 μg β-hCG core peptide or the α-hCG sub unit per day, and the pups were dosed through the mother's milk.

The unfractionated APL™ hCG preparation, PREGNYL™ (Organon) hCG preparation, purified β-core and phenol were also tested in certain assays. Phenol, which is an additive in the hCG APL™ preparation, was tested to control for any effect on cell growth or viral inhibition.

8.2. RESULTS

Fractionation of both the APL™ hCG preparation and the human early pregnancy urine resulted in a significant protein peak at approximately 158 kD with diminishing, but still detectable, protein in the rest of the fractions, even those containing small molecular weight material (Figures 6A and D). Fractions containing the hCG dimer (77 kD) and the β-hCG core (10 kD) were identified by immunoprecipitation using antibodies that specifically recognize these particular species, as described in the materials and methods section 8.1. The elution profile of the commercial hCG material was also plotted in comparison to the elution of

standard molecular weight markers (Figures 9A and B). Additionally, Fractions 61, 63, 64, 65 and 67 from the fractionation of the commercial hCG material was analyzed by MALDI-TOF mass spectrometry (Figures 10A-E, respectively).

8.2.1. EFFECT OF FRACTIONS OF COMMERCIAL hCG PREPARATIONS AND EARLY PREGNANCY URINE ON HIV-1 REPLICATION IN VITRO

The fractions of both the APL™ hCG preparation and the human early pregnancy urine were assayed for inhibition of HIV-1 IIID replication in PBMCs and PM-1 cells as described above.Many of the APL™ hCG preparation fractions exhibited significant inhibition of HIV-1 IIID replication (Figure 6C). In particular, fractions containing material of approximately 70 kD to approximately 2-3 kD exhibited HIV-1 inhibitory activity. The fractions effecting the highest percent inhibition of HIV-1 replication were fractions 62, 63, 65, and 73, with the three main peaks of activity eluting with apparent molecular weights of approximately 40 kD, approximately 15 kD, and approximately 2-3 kD, as determined by comparison with the elution of native hCG (77 kD) and β-core protein (10 kD).

The fractions of human early pregnancy urine were also assayed for ability to inhibit HIV-1 IIID replication in the PBMCs and the PM-1 cells. Again, several fractions had at least some HIV-1 replication-inhibitory activity. Fractions 64 and 67 caused more than twice the inhibition of HIV-1 IIID replication than any of the other fractions (Figure 6F). There were approximately two peaks of activity eluting from the gel filtration column with apparent molecular weights of approximately 15 kD and 3 kD, as determined by comparison with the elution of native hCG (77 D) and β-core protein (10 kD) identified by immunoassay.

Additionally, phenol had no effect on HIV-1 replication, demonstrating that the anti-HIV activity of the APL™ hCG is not due to the presence of phenol in the APL™ hCG preparation, and purified β-hCG core protein (the peptide of

amino acids to 6-40 of β-hCG linked via a disulfide bond to the peptide of amino acids 55-92 of β-hCG as depicted in Figure 4 (SEQ ID NO:2)) was also found not to inhibit HIV-1 replication (data not shown).

8.2.2. EFFECT OF FRACTIONS OF COMMERCIAL hCG AND EARLY PREGNANCY URINE ON KAPOSI'S SARCOMA CELL GROWTH IN VITRO

The fractions of APL™ hCG and human early pregnancy urine were also tested for inhibition of the proliferation of cultured Kaposi's Sarcoma cells. Figure 6B depicts the results of assays of the APL™ hCG fractions for inhibition of KS Y-1 cell growth. There were three major peaks of KS cell growth inhibitory activity which eluted from the gel filtration column with apparent molecular weights of approximately 40 kD, approximately 15 kD, and approximately 2-3 kD, as compared with the elutions of fractions containing hCG dimer (77 kD) and β-core protein (10 kD). A fraction containing material about the same size as the β-hCG core protein exhibited the highest level of inhibition; however, purified β-hCG core was found not to inhibit KS cell growth (data not shown).

Fractions of human early pregnancy urine were also assayed for inhibition of KS Y-1 cell growth. Fractions containing material which eluted from the gel filtration column with apparent molecular weights of approximately 15 kD and approximately 2-3 kD as compared with the elution of fractions containing hCG dimer (77 kD) and the β-hCG core (10 kD) as identified by immunoprecipitation assay were the most effective at inhibiting KS cell growth, with the approximately 15 kD fractions having the highest activity (Figure 6E).

8.2.3. EFFECT OF FRACTIONS OF A COMMERCIAL hCG PREPARATION AND EARLY PREGNANCY URINE ON hCG GENE EXPRESSION IN HIV-I TRANSGENIC MICE

Finally, fractions from the APL™ hCG and early pregnancy urine fractionation were tested for their ability to improve survival and to reduce HIV-I gene expression in HIV-I transgenic mice. Figure 1E presents results of administration of 200 μl of fraction 61 of the APL™ hCG fractionation (bar 4) and fraction 65 of the early pregnancy urine fractionation (bar 5). These two fractions, both of which are within the peak of anti-HIV and anti-KS activity that contains material with an apparent molecular weight of approximately 15 kD, significantly suppressed HIV-I gene expression in the HIV-I transgenic mice (as measured in the skin and the kidney) in comparison to PBS alone (bar 1), β-hCG core peptide (bar 2) and α-hCG subunit (bar 3).

8.3. CONCLUSION

The above-described experiments demonstrate that the factor(s) responsible for the anti-HIV and anti-KS activities can be further isolated from the hCG preparations by gel filtration on a SUPERDEX™ 200 gel filtration column. The factor(s) were fractionated from both the commercial APL™ hCG preparation and urine from women in early pregnancy (first trimester). The fractions of highest anti-HIV and anti-KS activity contained material eluting from the gel filtration column with an apparent molecular weights of approximately 40 kD, 15 kD and 2-3 kD. Although certain active fractions contained material of approximately the size of the β-hCG core protein (~10 kD), purified β-hCG core protein was found to have neither anti-HIV nor anti-KS activity. The fractions exhibiting anti-HIV and anti-KS activity in vitro also promoted weight gain in HIV-I transgenic mice. Furthermore, phenol, an additive in the APL™ hCG preparation, had no anti-HIV activity.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various references are cited herein, the disclosures of which are incorporated by reference in their entireties.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Gallo, Robert C.
Bryant, Joseph
Lunardi-Iskandar, Yanto

(ii) TITLE OF THE INVENTION: METHODS OF TREATMENT OF WASTING SYNDROME BASED ON ADMINISTRATION OF DERIVATIVES OF HUMAN CHORIONIC GONADOTROPIN

(iii) NUMBER OF SEQUENCES: 37

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 2.0

(vi) CURRENT APPLICATION DATA:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 539 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 26..520

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
AGACAAGGCA GGGGACGCAC CAAGG ATG GAG ATG TTC CAG GGG CTG CTG CTG      52
                            Met Glu Met Phe Gln Gly Leu Leu Leu
                            -20                 -15

TTG CTG CTG CTG AGC ATG GGC GGG ACA TGG GCA TCC AAG GAG CCG CTT     100
Leu Leu Leu Leu Ser Met Gly Gly Thr Trp Ala Ser Lys Glu Pro Leu
    -10                 -5                   1

CGG CCA CGG TGC CGC CCC ATC AAT GCC ACC CTG GCT GTG GAG AAG GAG     148
Arg Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu Ala Val Glu Lys Glu
                 10                  15                  20

GGC TGC CCC GTG TGC ATC ACC GTC AAC ACC ACC ATC TGT GCC GGC TAC     196
Gly Cys Pro Val Cys Ile Thr Val Asn Thr Thr Ile Cys Ala Gly Tyr
            25                  30                  35

TGC CCC ACC ATG ACC CGC GTG CTG CAG GGG GTC CTG CCG GCC CTG CCT     244
Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro
        40                  45                  50

CAG GTG GTG TGC AAC TAC CGC GAT GTG CGC TTC GAG TCC ATC CGG CTC     292
Gln Val Val Cys Asn Tyr Arg Asp Val Arg Phe Glu Ser Ile Arg Leu
    55                  60                  65

CCT GGC TGC CCG CGC GGC CTG AAC CCC GTG GTC TCC TAC GCC GTG GCT     340
Pro Gly Cys Pro Arg Gly Leu Asn Pro Val Val Ser Tyr Ala Val Ala
70                  75                  80                  85

CTC AGC TGT CAA TGT GCA CTC TGC CGC CGC AGC ACC ACT GAC TGC GGG     388
Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg Ser Thr Thr Asp Cys Gly
                 90                  95                 100

GGT CCC AAG GAC CAC CCC TTG ACC TGT GAT GAC CCC CGC TTC CAG GAC     436
Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp
            105                 110                 115

TCC TCT TCC TCA AAG GCC CCT CCC CCC AGC CTT CCA AGC CCA TCC CGA     484
Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg
        120                 125                 130

CTC CCG GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA TAAAGGCTTC          530
Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln
    135                 140                 145

TCAATCCGC                                                           539
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 165 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Glu Met Phe Gln Gly Leu Leu Leu Leu Leu Leu Leu Ser Met Gly
-20                 -15                 -10                  -5

Gly Thr Trp Ala Ser Lys Glu Pro Leu Arg Pro Arg Cys Arg Pro Ile
                 1               5                   10

Asn Ala Thr Leu Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr
         15                  20                  25

Val Asn Thr Thr Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val
     30                  35                  40

Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg
 45                  50                  55                      60

Asp Val Arg Phe Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Leu
                 65                  70                  75

Asn Pro Val Val Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu
             80                  85                  90

Cys Arg Arg Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu
         95                 100                 105

Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro
    110                 115                 120

Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr
125                 130                 135                     140

Pro Ile Leu Pro Gln
                145
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro Gln
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Leu Gln Gly Val Leu Pro Ala Leu Pro Gln
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Gly Val Leu Pro Ala Leu Pro
1               5
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular, linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Val Leu Gln Gly Val Leu Pro Ala Leu Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Val Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Leu Gln Gly Val Leu Pro Ala Leu Pro
1               5
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Gln Gly Val Leu Pro Ala Leu Pro
1               5
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Leu Gln Gly Val Leu Pro Ala Leu Pro Gln
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Gly Val Leu Pro Ala Leu Pro Gln
1               5
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Gly Val Leu Pro Ala Leu Pro Gln Val
1               5
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
Gly Val Leu Pro Ala Leu Pro Gln Val Val
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg Asp Val Arg
1               5                   10                  15
```

Phe Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Leu Asn Pro Val
20 25 30

Val Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg
35 40 45

Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp
50 55 60

Asp Pro Arg Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser
65 70 75 80

Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu
85 90 95

Pro Gln

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 88 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Asn Tyr Arg Asp Val Arg Phe Glu Ser Ile Arg Leu Pro Gly Cys Pro
1 5 10 15

Arg Gly Leu Asn Pro Val Val Ser Tyr Ala Val Ala Leu Ser Cys Gln
20 25 30

Cys Ala Leu Cys Arg Arg Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp
35 40 45

His Pro Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Ser Ser
50 55 60

Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro
65 70 75 80

Ser Asp Thr Pro Ile Leu Pro Gln
85

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro
1 5 10 15

```
Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr
            20                  25                  30

Pro Ile Leu Pro Gln
        35
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Cys Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Thr Cys Asp
1 5 10 15
Asp Pro Arg Phe Gln Asp Ser Ser
20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Leu Gln Gly Val Leu Pro
1 5 10 15
Ala Leu Pro Gln Val Val Cys
20

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Leu Thr Cys Asp Asp
1 5 10 15
Pro Arg Phe Gln Asp Ser Ser
20

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu Ala Val Glu Lys Glu Gly
 1               5                  10                  15
Cys Pro Val Cys Ile Thr Val Asn Thr Thr Ile Cys Ala Gly Tyr Cys
            20                  25                  30
Pro Thr
```

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg Asp
 1               5                  10                  15
Val Arg Phe Glu
            20
```

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```
Val Leu Pro Ala Leu Pro Gln Val Val
 1               5
```

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
Cys Val Ala Gln Pro Gly Pro Gln Val Leu Leu Val Leu Cys
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```
Cys Val Ala Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys
 1              5                   10                  15
```

WHAT IS CLAIMED IS:

1. A method of treating or preventing wasting syndrome in a subject in need of such treatment or prevention comprising administering to the subject an amount of a preparation comprising hCG or β-hCG effective to treat or prevent wasting syndrome.

2. The method of claim 1 in which the subject is a human.

3. The method of claim 1 in which the wasting syndrome is associated with a viral infection, cancer or chronic cardiovascular disease.

4. The method of claim 3 in which the wasting syndrome is associated with HIV infection.

5. The method of claim 1 in which the wasting syndrome is associated with chemotherapy or radiation therapy.

6. The method of claim 1 in which at least 10,000 units, at least 20,000 units or at least 45,000 units of hCG is administered per week.

7. A method of treating or preventing wasting syndrome in a subject in need of such treatment or prevention comprising administering to the subject an amount of a purified protein effective to treat or prevent wasting syndrome, which protein comprises one or more portions of the amino acid sequence of β-hCG, a peptide having an amino acid sequence consisting of said one or more portions being active to treat or prevent wasting syndrome.

8. The method of claim 7 in which the amino acid sequence of at least one of said one or more portions is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53,

44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25, and 33-35 respectively), as depicted in Figure 4 (a portion of SEQ ID NO:2).

9. The method of claim 7 in which the purified protein comprises two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 4 (SEQ ID NO:2), in which said portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion.

10. The method of claim 9, in which the amino acid sequence of said protein is selected from the group consisting of β-hCG amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 109-119 (SEQ ID NO:7), β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 45-57 (SEQ ID NO:6), and β-hCG amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 108-119 (SEQ ID NO:29) as depicted in Figure 4 (SEQ ID NO:2).

11. The method of claim 7 in which the protein is N-acetylated and has a C-terminal amide.

12. The method of claim 7 in which the wasting syndrome is associated with a viral infection, cancer or chronic cardiovascular disease.

13. The method of claim 12 in which the wasting syndrome is associated with HIV infection.

14. The method of claim 7 in which the wasting syndrome is associated with chemotherapy or radiation therapy.

15. A method of treating or preventing wasting syndrome in a subject in need of such treatment or prevention comprising administering to the subject an amount of a purified derivative of a protein effective to treat or prevent wasting syndrome, which protein comprises one or more portions of the amino acid sequence of β-hCG, said derivative being active to treat or prevent wasting syndrome.

16. The method of claim 15 in which the amino acid sequence of at least one of said one or more portions is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25, and 33-35 respectively), as depicted in Figure 4 (a portion of SEQ ID NO:2).

17. The method of claim 15 in which the purified derivative comprises two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 4 (SEQ ID NO:2), in which said portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion.

18. The method of claim 17, in which the amino acid sequence of said protein is selected from the group consisting of β-hCG amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 109-119 (SEQ ID NO:7), β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 45-57 (SEQ ID NO:6), and β-hCG amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 108-119 (SEQ ID NO:29) as depicted in Figure 4 (SEQ ID NO:2).

19. The method of claim 7 or 15 in which the subject is a human.

20. The method of claim 7 or 15 in which the amino acid sequence of said protein consists of amino acid numbers 45-57 (SEQ ID NO:6) as depicted in Figure 4 (a portion of SEQ ID NO:2).

21. The method of claim 15 in which the derivative contains one or more D-amino acids or one or more non-classical amino acids.

22. A method of treating or preventing wasting syndrome in a subject in need of such treatment or prevention comprising administering to the subject an amount of a circularized protein effective to treat or prevent wasting syndrome, the amino acid sequence of which protein consists of one or more portions of the sequence of β-hCG as depicted in Figure 4 (a portion of SEQ ID NO:2), in which a cysteine residue is inserted or substituted for a different amino acid residue in at least one of said one or more portions of said sequence, said one or more portions of said sequence containing a second cysteine residue, and in which a disulfide bond is formed between the inserted or substituted cysteine residue and the second cysteine residue present in said one or more portions of said sequence, said protein being active to treat or prevent wasting syndrome.

23. The method of claim 22 in which the amino acid sequence of at least one portion is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25, and 33-35 respectively), as depicted in Figure 4 (a portion of SEQ ID NO:2).

24. The method of claim 22 in which the circularized protein has an amino acid sequence which consists of β-hCG amino acid numbers 44-57 (SEQ ID NO:12) as depicted in Figure 4 (a portion of SEQ ID NO:2), with cysteine substituted for valine at position 44.

25. The method of claim 22 in which said circularized protein consists of two or more at least five amino acid, non-naturally contiguous portions of the sequence of β-hCG as depicted in Figure 4 (SEQ ID NO:2), in which the portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion.

26. The method of claim 25, in which the first portion consists of β-hCG amino acid numbers 45-57 (SEQ ID NO:6) and the second portion consists of β-hCG amino acid numbers 110-119 (SEQ ID NO:27) as depicted in Figure 4 (SEQ ID NO:2); and in which a disulfide bond is formed between the cysteine residues at a amino acids 57 and 110 of said portions.

27. A method of treating or preventing wasting syndrome in a subject in need of such treatment or prevention comprising administering to the subject an amount of a purified protein effective to treat or prevent wasting syndrome, in which the protein has an amino acid sequence which consists of one or more portions of the sequence of β-hCG as depicted in Figure 4 (SEQ ID NO:2) in which one or more residues in at least one of said one or more portions of said sequence are substituted by an amino acid or amino acid analog having a side chain with an amino or carboxyl group, said amino or carboxyl group forming a peptide bond with a second sequence of one or more amino acids, said protein being active to treat or prevent wasting syndrome.

28. The method of claim 27 in which said at least one portion of said sequence consists of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53,

44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25, and 33-35 respectively), as depicted in Figure 4 (a portion of SEQ ID NO:2).

29. The method of claim 27 in which said protein consists of amino acid numbers 45-57 (SEQ ID NO:6) as depicted in Figure 4 (a portion of SEQ ID NO:2), and in which substitutions by said amino acid or amino acid analog occur at residues 47 and 51 of said portion.

30. The method of claim 27 in which said one or more residues are each substituted by a diaminobutyric acid residue and the side chain amino group of said diaminobutyric acid residue is peptide bonded to a sequence of one or more proline residues.

31. The method of claim 29 in which the residues at positions 47 and 51 of said portion are each substituted by a diaminobutyric acid residue and the side chain amino group of said diaminobutyric acid residue is peptide bonded to a proline residue.

32. A method of treating or preventing wasting syndrome in a subject in need of such treatment or prevention comprising administering to the subject an amount of a circularized protein effective to treat or prevent wasting syndrome, the amino acid sequence of which protein consists of one or more portions of the sequence of β-hCG as depicted in Figure 4 (SEQ ID NO:2) in which a cysteine residue is inserted or substituted for a different amino acid residue in at least one of said one or more portions of said sequence, said one or more portions of said sequence containing a second cysteine residue, and in which a disulfide bond is formed between the inserted or substituted cysteine residue and the second cysteine residue present in said one or more portions of said sequence, and also in which one or more

residues in at least one of said one or more portions of said sequence are substituted by an amino acid or amino acid analog having a side chain with an amino or carboxyl group, said amino or carboxyl group forming a peptide bond with a second sequence of one or more amino acids, said protein being active to treat or prevent wasting syndrome.

33. The method of claim 32 in which the circularized protein has an amino acid sequence which consists of β-hCG amino acid numbers 44-57 (SEQ ID NO:12) as depicted in Figure 4 (a portion of SEQ ID NO:2), with cysteine substituted for valine at position 44, and in which the residues at positions 47 and 51 of said portion are each substituted by a diaminobutyric acid residue, and the side chain amino group of said diaminobutyric acid residue is peptide bonded to a proline residue.

34. A method of treating or preventing wasting syndrome in a subject in need of such treatment or prevention comprising administering to the subject an amount of a purified protein effective to treat or prevent wasting syndrome, which protein (a) comprises a portion of the amino acid sequence of β-hCG, a peptide having an amino acid sequence consisting of said portion being active to treat or prevent wasting syndrome; and (b) lacks β-hCG amino acids contiguous to said portion.

35. The method of claim 34 in which the portion consists of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25, and 33-35 respectively), as depicted in Figure 4 (a portion of SEQ ID NO:2).

36. The method of claim 35 in which the portion consists of amino acid numbers 45-57 (SEQ ID NO:6) as depicted in Figure 4 (a portion of SEQ ID NO:2).

37. The method of claim 34 in which the subject is a human.

38. The method of claim 34 in which the protein is a fusion protein, said fusion protein comprising said portion joined via a peptide bond to a protein sequence of a protein different from β-hCG.

39. The method of claim 34 in which the wasting syndrome is associated with a viral infection, cancer or chronic cardiovascular disease.

40. The method of claim 39 in which the wasting syndrome is associated with HIV infection.

41. The method of claim 34 in which the wasting syndrome is associated with chemotherapy or radiation therapy.

42. A method of treating or preventing wasting syndrome in a human subject in need of such treatment or prevention comprising administering to the subject an amount of the first composition comprising one or more first components of a second composition comprising a sample of native hCG or native β-hCG, said first composition being separated from other components of the hCG or β-hCG sample, said first components having anti-wasting activity, and said second composition having anti-wasting activity, and said native hCG or native β-hCG not being purified to homogeneity in said second composition.

43. The method of claim 42 in which said first components have an approximate apparent molecular weight

selected from the group consisting of 40 kD, 15 kD and 3 kD, wherein said apparent molecular weight is determined by elution from a gel filtration sizing column relative to the elution of a native hCG heterodimer, having a molecular weight of 77 kD, and a β-hCG core protein, having a molecular weight of 10 kD.

44. A method of treating or preventing wasting syndrome in a human subject in need of such treatment or prevention comprising administering to the subject an amount of a first composition effective to treat or prevent wasting syndrome, said first composition produced by a process comprising the following steps:

(a) subjecting a second composition comprising native hCG or native β-hCG, said second composition having anti-wasting activity, said native hCG or native β-hCG not being purified to homogeneity in said second composition, to a size fractionation procedure; and

(b) recovering fractions having anti-wasting activity.

45. The method of claim 44, in which the recovered fractions contain material having an approximate apparent molecular weight selected from the group consisting of 40 kD, 15 kD and 3 kD, wherein said apparent molecular weight is determined by elution from a gel filtration sizing column relative to the elution of a native hCG heterodimer, having a molecular weight of 77 kD, and a β-hCG core protein, having a molecular weight of 10 kD.

46. A method for screening a preparation comprising hCG or an hCG α chain or hCG β chain or a derivative of hCG or of said alpha or beta chain or a fraction of a source of native hCG or native β-hCG, for anti-wasting activity comprising assaying said preparation for the ability to promote weight gain in an animal model that exhibits a wasting syndrome.

47. The method of claim 46 in which the preparation is screened by a method comprising measuring the weight of an offspring of an HIV-1 transgenic mouse, which offspring has been exposed to the preparation; and comparing the weight of the offspring which has been exposed to the preparation with the weight of an offspring not so exposed, wherein a greater weight in said exposed offspring indicates that the preparation has anti-wasting activity.

48. The method of claim 46 in which the preparation is screened by a method comprising measuring the change in weight of an SIV infected monkey which has been exposed to the preparation; and comparing the change in weight of the monkey which has been exposed to the preparation to the change in weight of a monkey which has not been so exposed, wherein a greater weight increase or smaller weight loss in said exposed monkey indicates that the preparation has anti-wasting activity.

49. A pharmaceutical composition comprising an amount of a preparation comprising β-hCG effective for treatment of wasting syndrome; and a pharmaceutically acceptable carrier.

50. The pharmaceutical composition of claim 49 which is formulated as a controlled release formulation.

51. A pharmaceutical composition comprising an amount effective for treatment of wasting syndrome of a purified protein, the amino acid sequence of which protein is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-57, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively), as depicted in Figure 4 (a portion of SEQ ID NO:2); and a pharmaceutically acceptable carrier.

52. The pharmaceutical composition of claim 51 in which the amino acid sequence of the protein consists of amino acid numbers 45-57 (SEQ ID NO:6) as depicted in Figure 4 (a portion of SEQ ID NO:2).

53. A pharmaceutical composition comprising a therapeutically effective amount of a purified protein effective to treat or prevent wasting syndrome, the amino acid sequence of which comprises two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 4 (SEQ ID NO:2), in which the portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion; and a pharmaceutically acceptable carrier.

54. The pharmaceutical composition of claim 51 or 53 which is formulated as a controlled release formulation.

55. The pharmaceutical composition of claim 53, in which the amino acid sequence of said protein is selected from the group consisting of β-hCG amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 109-119 (SEQ ID NO:7), β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 45-57 (SEQ ID NO:6), and β-hCG amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 108-119 (SEQ ID NO:29) as depicted in Figure 4 (SEQ ID NO:2).

56. The pharmaceutical composition of claim 53 in which the protein is a fusion protein, said fusion protein comprising at least one of said portions of the β-hCG amino acid sequence joined via a peptide bond to a sequence of a protein different from β-hCG.

57. The pharmaceutical composition of claim 51 or 53 in which the protein is N-acetylated and has a C-terminal amide.

58. A pharmaceutical composition comprising an amount effective for treatment of wasting syndrome of a derivative of a protein, the amino acid sequence of which protein is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively), as depicted in Figure 4 (a portion of SEQ ID NO:2); and a pharmaceutically acceptable carrier.

59. A pharmaceutical composition comprising a therapeutically effective amount of a derivative of a protein, the amino acid sequence of which protein comprises two or more at least five amino acid, non-naturally contiguous portions of the amino acid sequence of β-hCG as depicted in Figure 4 (SEQ ID NO:2), in which the portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion, said derivative being active to treat or prevent wasting syndrome; and a pharmaceutically acceptable carrier.

60. The pharmaceutical composition of claim 59, in which the amino acid sequence of said protein is selected from the group consisting of β-hCG amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 109-119 (SEQ ID NO:7), β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 45-57 (SEQ ID NO:6), and β-hCG amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of β-hCG amino acids 108-119 (SEQ ID NO:29) as depicted in Figure 4 (SEQ ID NO:2).

61. The method of claim 58 or 59 in which the derivative consists of one or more D-amino acids or one or more non-classical amino acids.

62. The pharmaceutical composition of claim 58 or 59 which is formulated as a controlled release formulation.

63. A pharmaceutical composition comprising an amount effective for treatment of a wasting syndrome of a circularized protein, the amino acid sequence of which consists of one or more portions of the sequence of β-hCG as depicted in Figure 4 (a portion of SEQ ID NO:2) in which a cysteine residue is inserted or substituted for a different amino acid residue in at least one of said one or more portions of said sequence, said one or more portions of said sequence containing a second cysteine residue, and in which a disulfide bond is formed between the inserted or substituted cysteine residue and the second cysteine residue present in said one or more portions of said sequence, said protein being active to treat or prevent wasting syndrome; and a pharmaceutically acceptable carrier.

64. The pharmaceutical composition of claim 63 in which the sequence of at least one portion is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-57, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively), as depicted in Figure 4 (a portion of SEQ ID NO:2).

65. The pharmaceutical composition of claim 63 in which the circularized protein has an amino acid sequence which consists of β-hCG amino acid numbers 44-57 (SEQ ID NO:12) as depicted in Figure 4 (a portion of SEQ ID NO:2), with cysteine substituted for valine at position 44.

66. The pharmaceutical composition of claim 63, in which the amino acid sequence of said circularized protein consists of two or more at least five amino acid, non-naturally contiguous portions of the β-hCG sequence as depicted in Figure 4 (SEQ ID NO:2) in which the portions are linked via a peptide bond between the N-terminus of a first said portion and the C-terminus of a second said portion.

67. The pharmaceutical composition of claim 66, in which the first portion consists of β-hCG amino acids 45-57 (SEQ ID NO:6) and the second portion consists of β-hCG amino acids 110-119 (SEQ ID NO:27) as depicted in Figure 4 (SEQ ID NO:2); and in which a disulfide bond is formed between the cysteine residue at amino acids 57 and 110 of said portions.

68. A pharmaceutical composition comprising an amount effective for treatment of a wasting syndrome of a purified protein, the amino acid sequence of which protein consists of one or more portions of the sequence of β-hCG as depicted in Figure 4 (SEQ ID NO:2), in which one or more residues in at least one of said one or more portions are substituted by an amino acid or amino acid analog having a side chain with an amino or carboxyl group, said amino or carboxyl group forming a peptide bond with a second sequence of one or more amino acids, said purified protein being active to treat or prevent wasting syndrome; and a pharmaceutically acceptable carrier.

69. The pharmaceutical composition of claim 68 in which said at least one portion of said sequence has a sequence selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-57, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively), as depicted in Figure 4 (a portion of SEQ ID NO:2).

70. The pharmaceutical composition of claim 68 in which said protein consists of amino acid numbers 45-57 (SEQ ID NO:6) as depicted in Figure 4 (a portion of SEQ ID NO:2), and in which substitutions by said amino acid or amino acid analog occur at residues 47 and 51 of said portion.

71. The pharmaceutical composition of claim 68 in which said one or more residues are each substituted by a diaminobutyric acid residue and the side chain amino group of said diaminobutyric acid residue is peptide bonded to a sequence of one or more proline residues.

72. The pharmaceutical composition of claim 70 in which the residues at positions 47 and 51 of said portion are each substituted by a diaminobutyric acid residue and the side chain amino group of said diaminobutyric acid residue is peptide bonded to a proline residue.

73. A pharmaceutical composition comprising an amount effective for treatment of a wasting syndrome of a circularized protein, the amino acid sequence of which consists of one or more portions of the sequence of β-hCG as depicted in Figure 4 (a portion of SEQ ID NO:2), in which a cysteine residue is inserted or substituted for a different amino acid residue in at least one of said one or more portions of said sequence, said one or more portions of said sequence containing a second cysteine residue, and in which a disulfide bond is formed between the inserted or substituted cysteine residue and the second cysteine residue present in said one or more portions of said sequence, and also in which one or more residues in at least one of said one or more portions of said sequence are substituted by an amino acid or amino acid analog having a side chain with an amino or carboxyl group, said amino or carboxyl group forming a peptide bond with a second sequence of one or more amino acids, said protein being active to treat or prevent wasting syndrome; and a pharmaceutically acceptable carrier.

74. The pharmaceutical composition of claim 73 in which said protein consists of β-hCG amino acid numbers 44-57 (SEQ ID NO:12) as depicted in Figure 4 (a portion of SEQ ID NO:2), and in which cysteine is substituted for valine at position 44, and in which the residues at positions 47 and 51 of said portion are each substituted by a diaminobutyric acid residue, and the side chain amino group of said diaminobutyric acid residue is peptide bonded to a proline residue.

75. A pharmaceutical composition comprising an amount effective for treatment of wasting syndrome of a purified protein, which protein (a) comprises a portion of the amino acid sequence of β-hCG, a peptide having an amino acid sequence consisting of said portion being active to treat or prevent wasting syndrome, and (b) lacks β-hCG amino acids contiguous to said portion; and a pharmaceutically acceptable carrier.

76. The pharmaceutical composition of claim 75 in which said portion of said sequence consists of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 and 48-56 (SEQ ID NOS:3-25 and 33-35, respectively), as depicted in Figure 4 (a portion of SEQ ID NO:2).

77. The pharmaceutical composition of claim 76 in which said portion of said sequence consists of amino acid numbers 45-57 (SEQ ID NO:6) as depicted in Figure 4 (a portion of SEQ ID NO:2).

78. The pharmaceutical composition of claim 75 in which the protein is a fusion protein, said fusion protein comprising said portion joined via a peptide bond to a protein sequence of a protein different from β-hCG.

79. A pharmaceutical composition comprising a therapeutically effective amount of a first composition effective to treat or prevent wasting syndrome, said first composition comprising one or more first components of a second composition comprising a sample of native hCG or native β-hCG, said first components being separated from other components of the hCG or β-hCG sample, said first components having anti-wasting activity, and said second composition having anti-wasting activity, and said native hCG or native β-hCG not being purified to homogeneity in said second composition; and a pharmaceutically acceptable carrier.

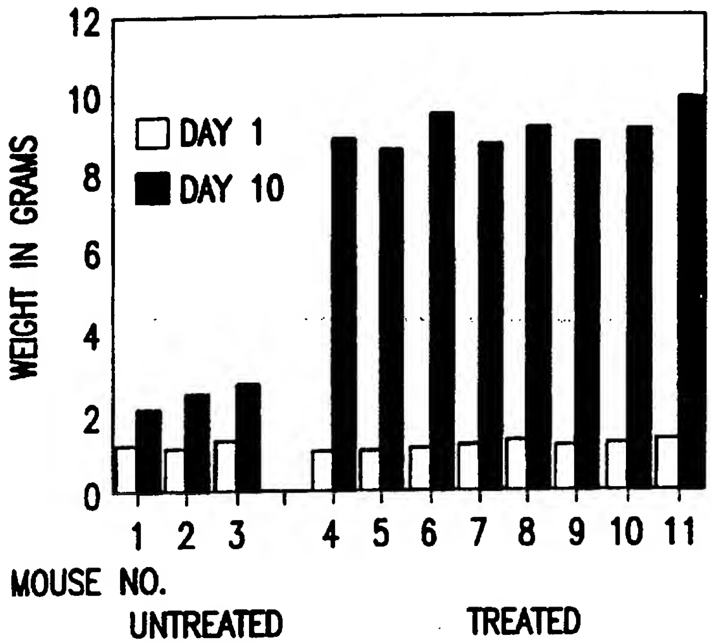
80. The pharmaceutical composition of claim 79 in which said first components are separated from said other components by sizing column chromatography.

81. The pharmaceutical composition of claim 80 in which said sizing column chromatography is performed using a SUPERDEX™ 200 column.

82. The pharmaceutical composition of claim 80 in which said first components have an approximate apparent molecular weight selected from the group consisting of 40 kD, 15 kD and 3 kD, wherein said apparent molecular weight is determined by elution from a gel filtration sizing column relative to the elution of a native hCG heterodimer, having a molecular weight of 77 kD, and a β-hCG core protein, having a molecular weight of 10 kD.

83. A pharmceutical composition comprising a therapeutically effective amount of a first composition effective to treat or prevent wasting syndrome, said first composition produced by a process comprising the following steps:

(a) subjecting a second composition comprising native hCG or native β-hCG, said second composition


12
10
8
6
4
2
0
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DAY 10
1 2 3 4 5 6 7 8 9 10 11
MOUSE NO.
UNTREATED
TREATED

FIG.1A

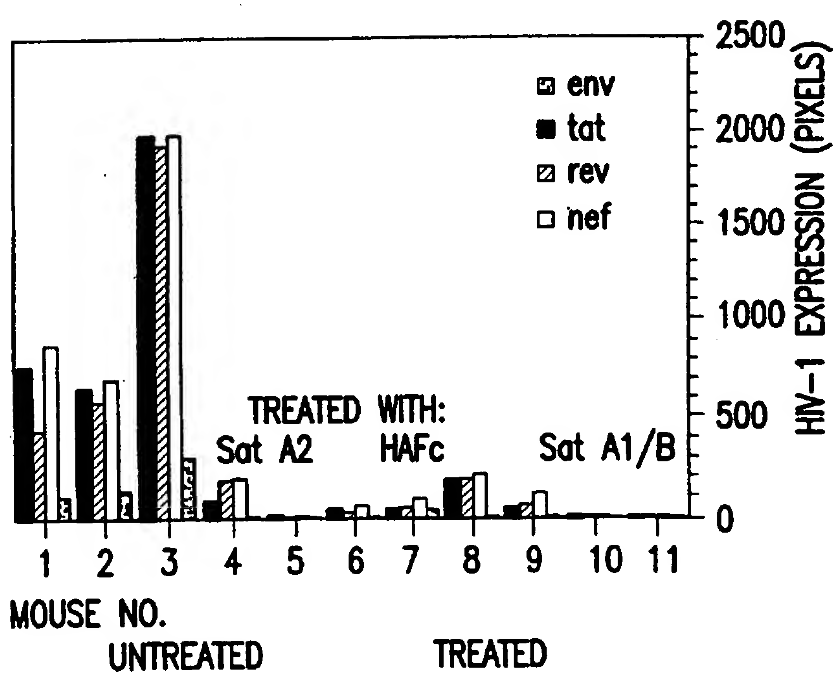

env
tat
rev
nef
2500
2000
1500
1000
500
0
HIV-1 EXPRESSION (PIXELS)
TREATED WITH:
Sat A2
HAFc
Sat A1/B
1 2 3 4 5 6 7 8 9 10 11
MOUSE NO.
UNTREATED
TREATED

FIG.1B

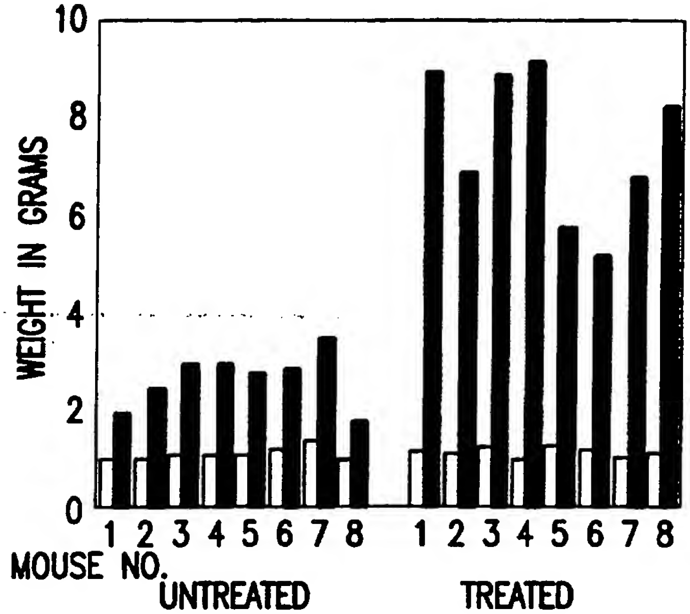

10
8
6
4
2
0
WEIGHT IN GRAMS
1 2 3 4 5 6 7 8 1 2 3 4 5 6 7 8
MOUSE NO.
UNTREATED
TREATED

FIG.1C

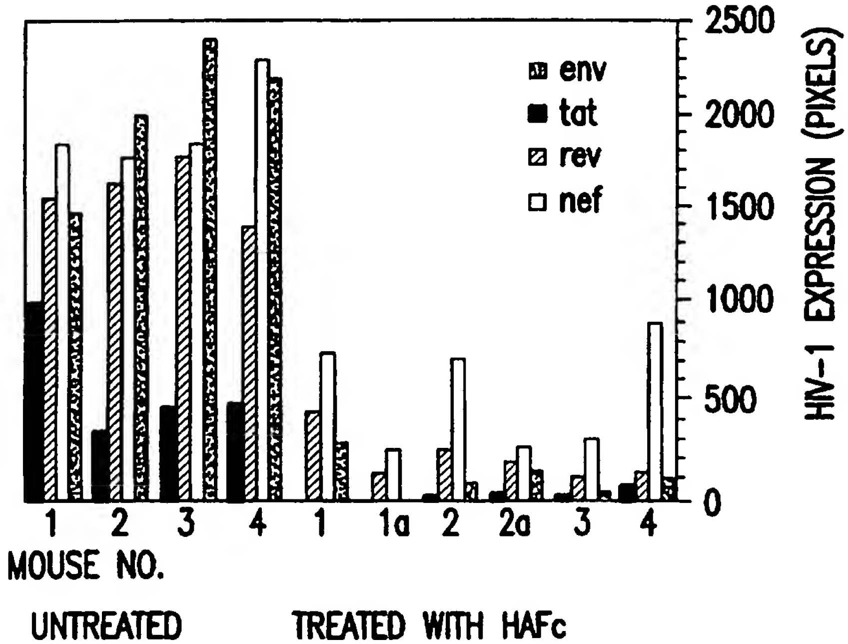

env
tat
rev
nef
2500
2000
1500
1000
500
0
HIV-1 EXPRESSION (PIXELS)
1 2 3 4 1 1a 2 2a 3 4
MOUSE NO.
UNTREATED
TREATED WITH HAFc

FIG.1D

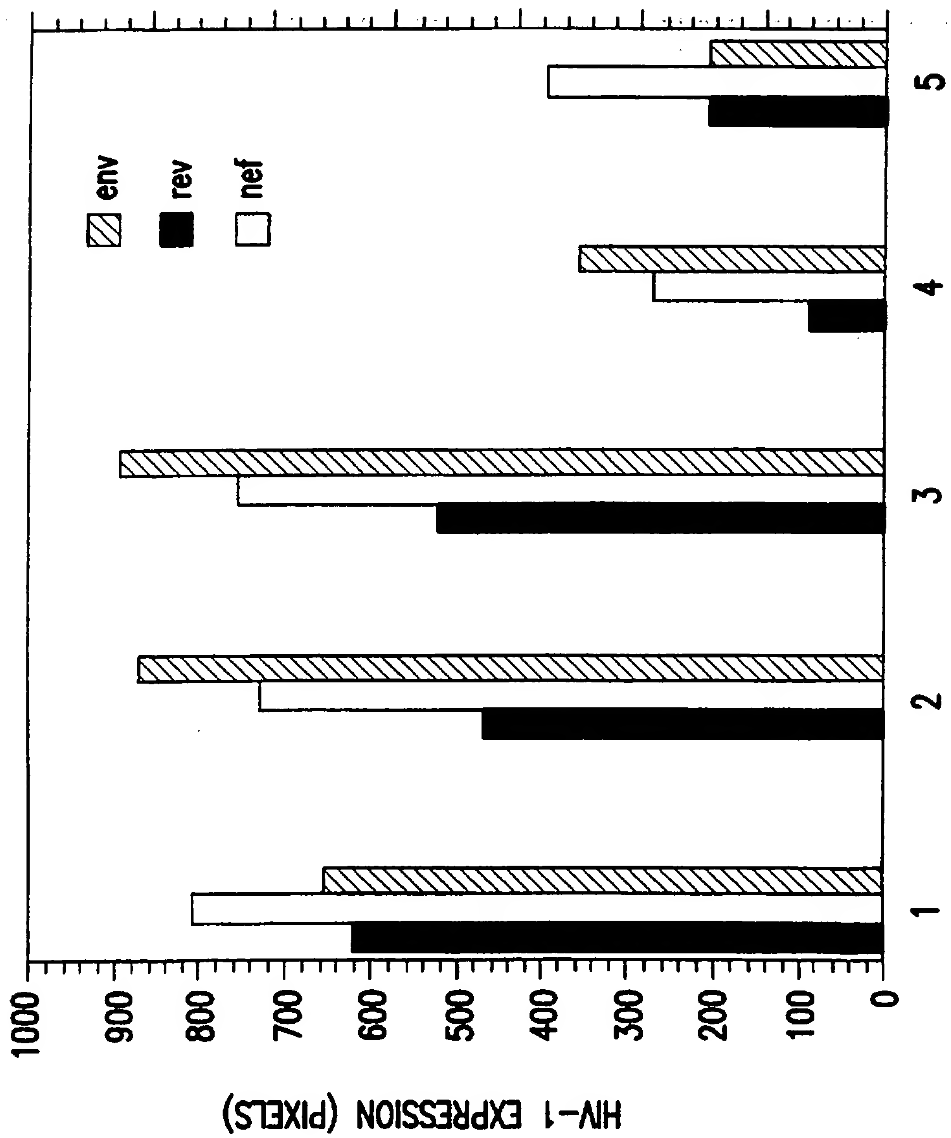
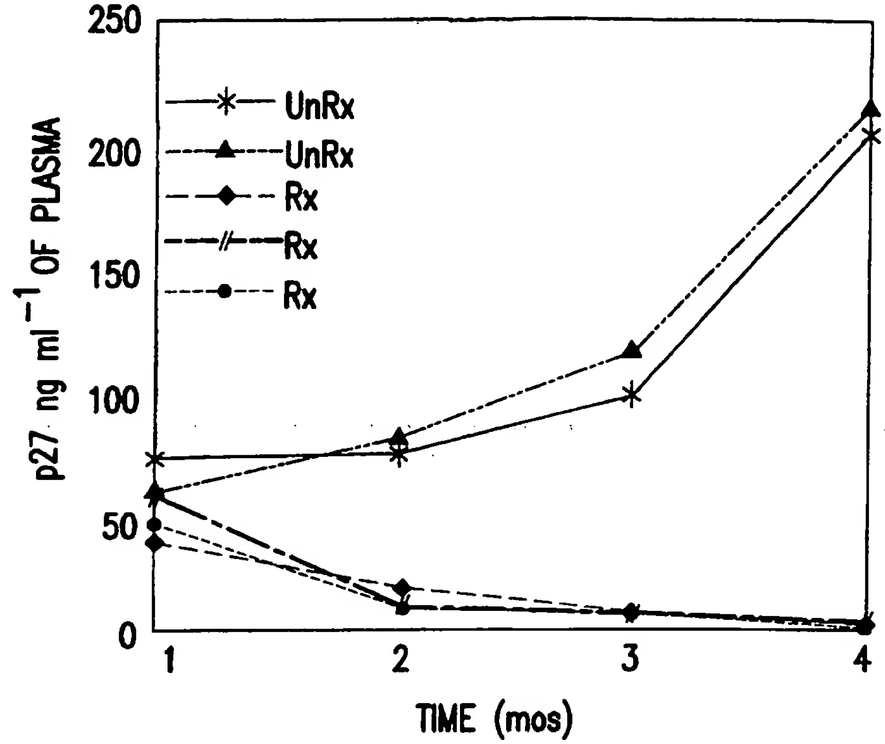

env
rev
nef
HIV-1 EXPRESSION (PIXELS)
1000
900
800
700
600
500
400
300
200
100
0
1
2
3
4
5

FIG.1E


250
200
150
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50
0
p27 ng ml −1 OF PLASMA
UnRx
UnRx
Rx
Rx
Rx
1
2
3
4
TIME (mos)

FIG.2A

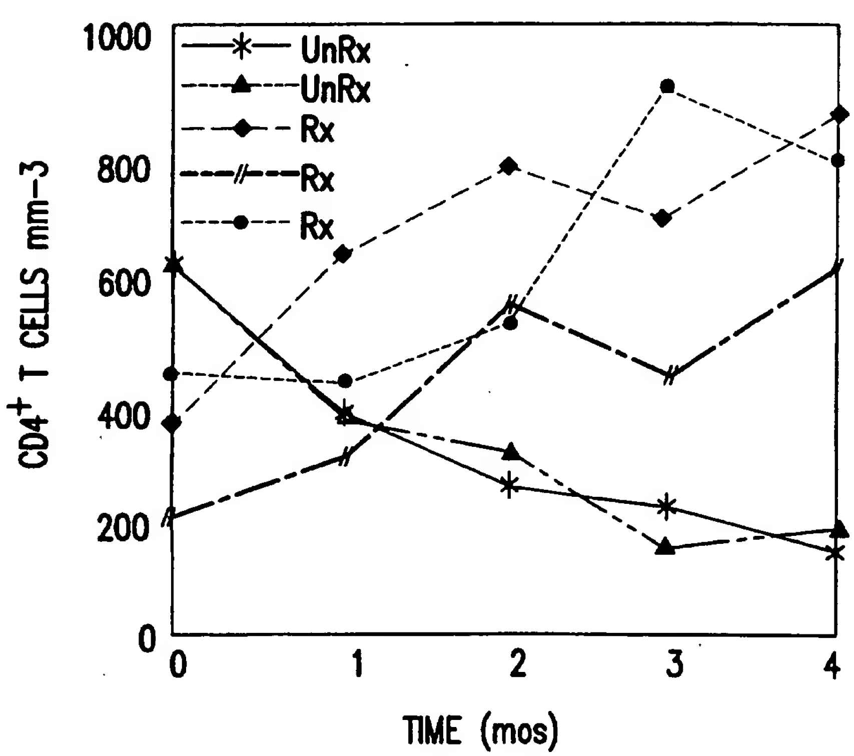
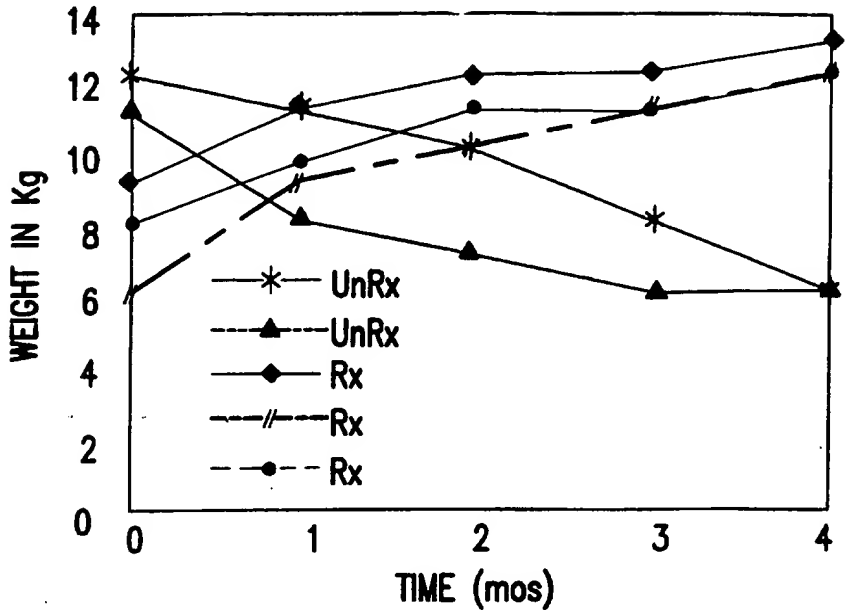
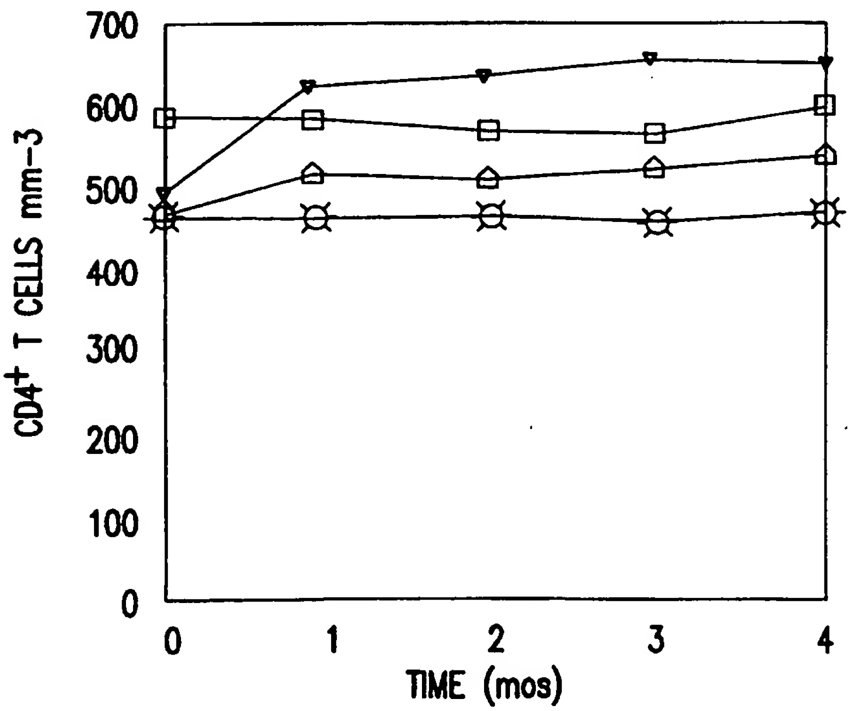

1000
800
600
400
200
0
CD4+ T CELLS mm-3
UnRx
UnRx
Rx
Rx
Rx
0
1
2
3
4
TIME (mos)

FIG.2B



14
12
10
8
6
4
2
0
WEIGHT IN Kg
UnRx
UnRx
Rx
Rx
Rx
0
1
2
3
4
TIME (mos)

FIG.2C



700
600
500
400
300
200
100
0
CD4+ T CELLS mm-3
0
1
2
3
4
TIME (mos)

FIG.2D

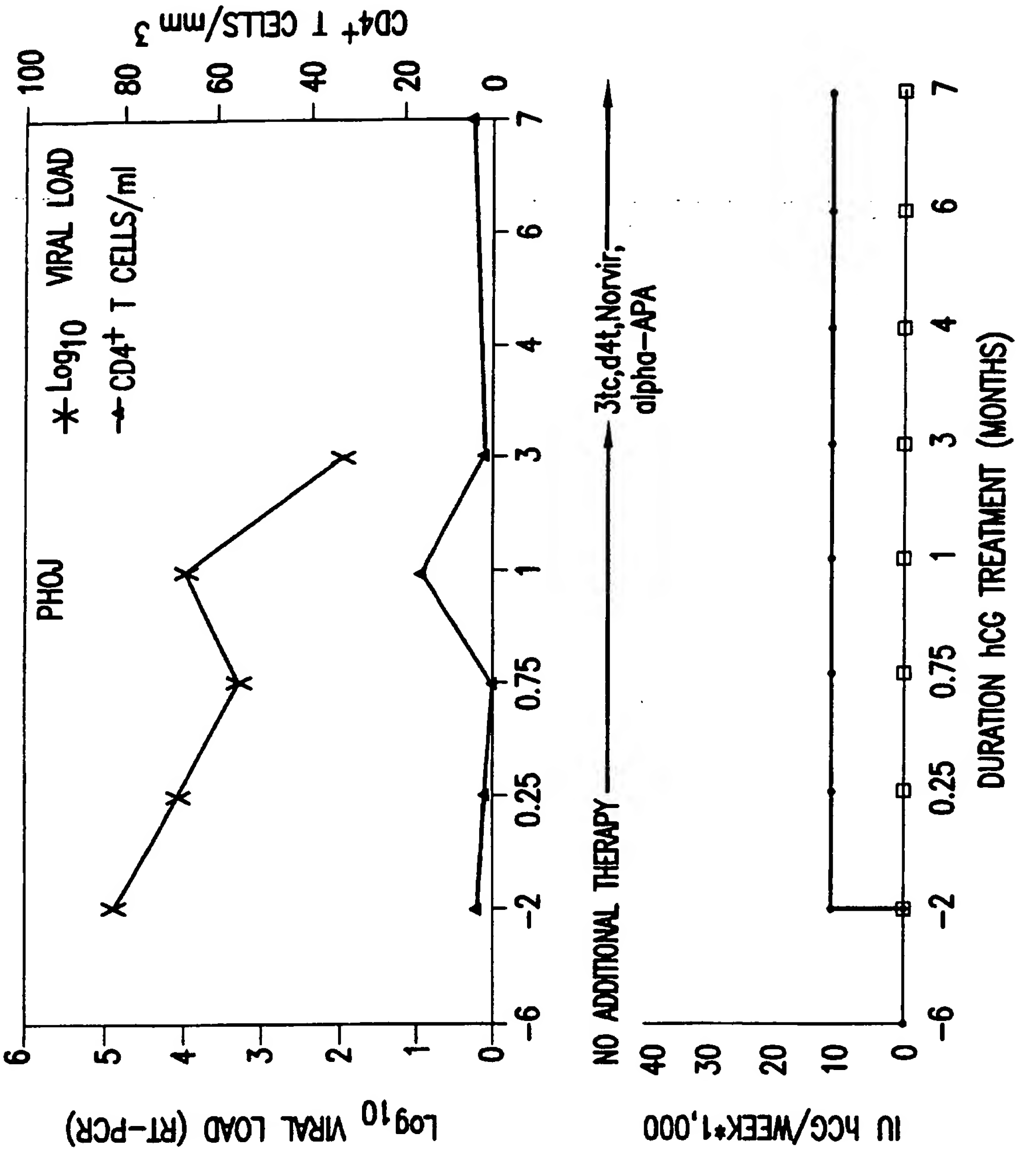

PHOJ
Log10 VIRAL LOAD
CD4+ T CELLS/ml
Log10 VIRAL LOAD (RT-PCR)
CD4+ T CELLS/mm 3
0
1
2
3
4
5
6
20
40
60
80
100
-6
-2
0.25
0.75
1
3
4
6
7
NO ADDITIONAL THERAPY
3tc,d4t,Norvir, alpha-APA
IU hCG/WEEK*1,000
10
30
DURATION hCG TREATMENT (MONTHS)

FIG.3A

FIG.3B

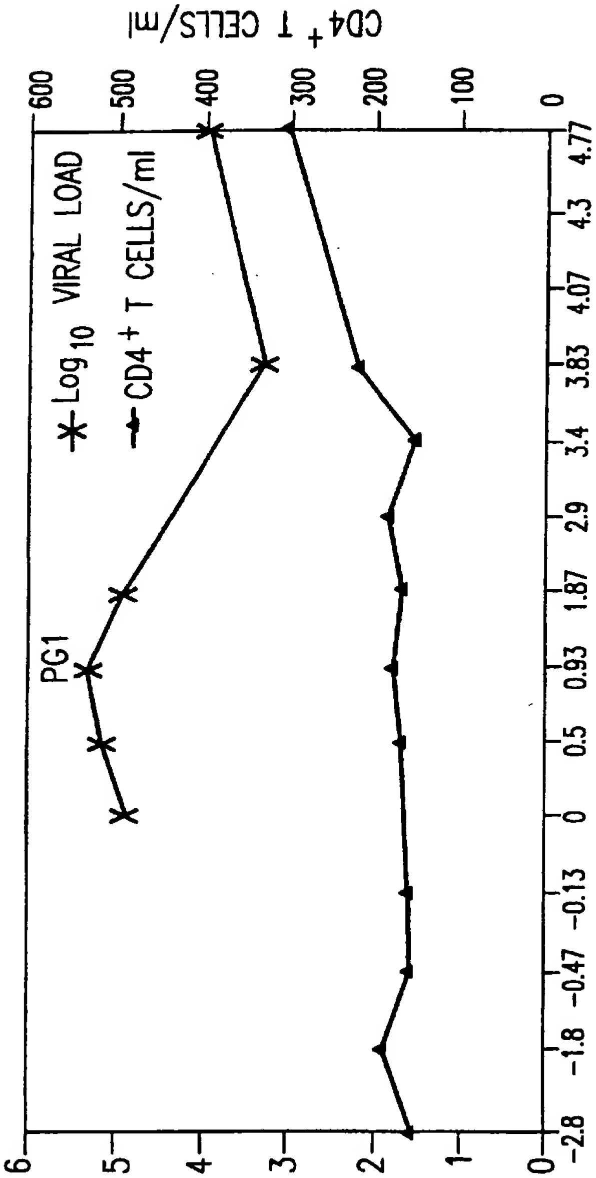

Log10 VIRAL LOAD
CD4+ T CELLS/ml
PG1
Log10 VIRAL LOAD (ROCHE AMPLICOR)
CD4+ T CELLS/ml
3tc, ddi
-2.8 -1.8 -0.47 -0.13 0 0.5 0.93 1.87 2.9 3.4 3.83 4.07 4.3 4.77
0 1 2 3 4 5 6
0 100 200 300 400 500 600

FIG.3C

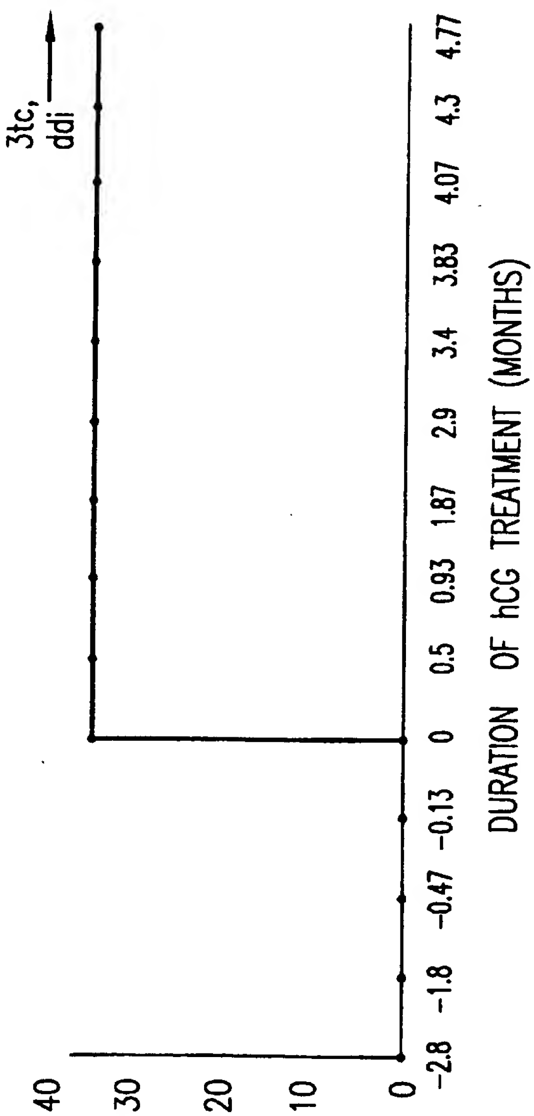

IU hCG/WEEK*1,000
0 10 20 30 40
-2.8 -1.8 -0.47 -0.13 0 0.5 0.93 1.87 2.9 3.4 3.83 4.07 4.3 4.77
DURATION OF hCG TREATMENT (MONTHS)

FIG.3D

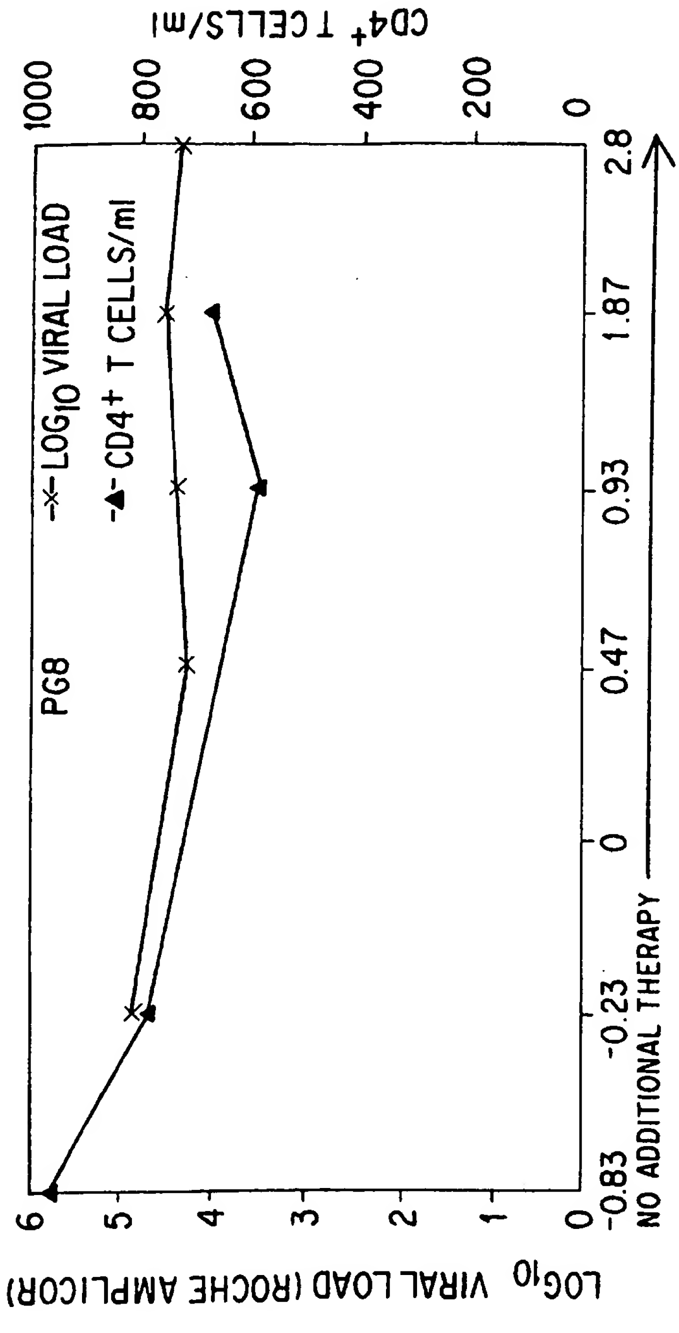

PG8
-x- LOG10 VIRAL LOAD
-▲- CD4+ T CELLS/ml
LOG10 VIRAL LOAD (ROCHE AMPLICOR)
CD4+ T CELLS/ml
NO ADDITIONAL THERAPY
-0.83
-0.23
0
0.47
0.93
1.87
2.8

FIG.3E

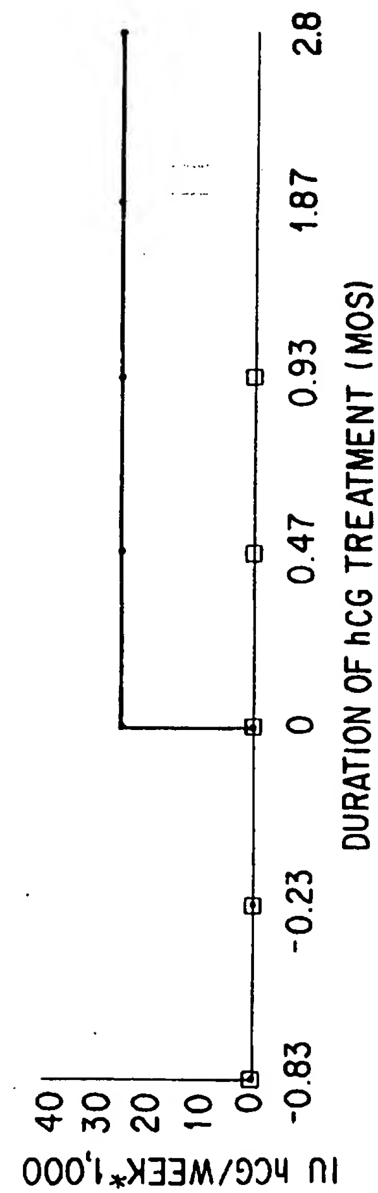

IU hCG/WEEK*1,000
DURATION OF hCG TREATMENT (MOS)
-0.83
-0.23
0
0.47
0.93
1.87
2.8

FIG.3F

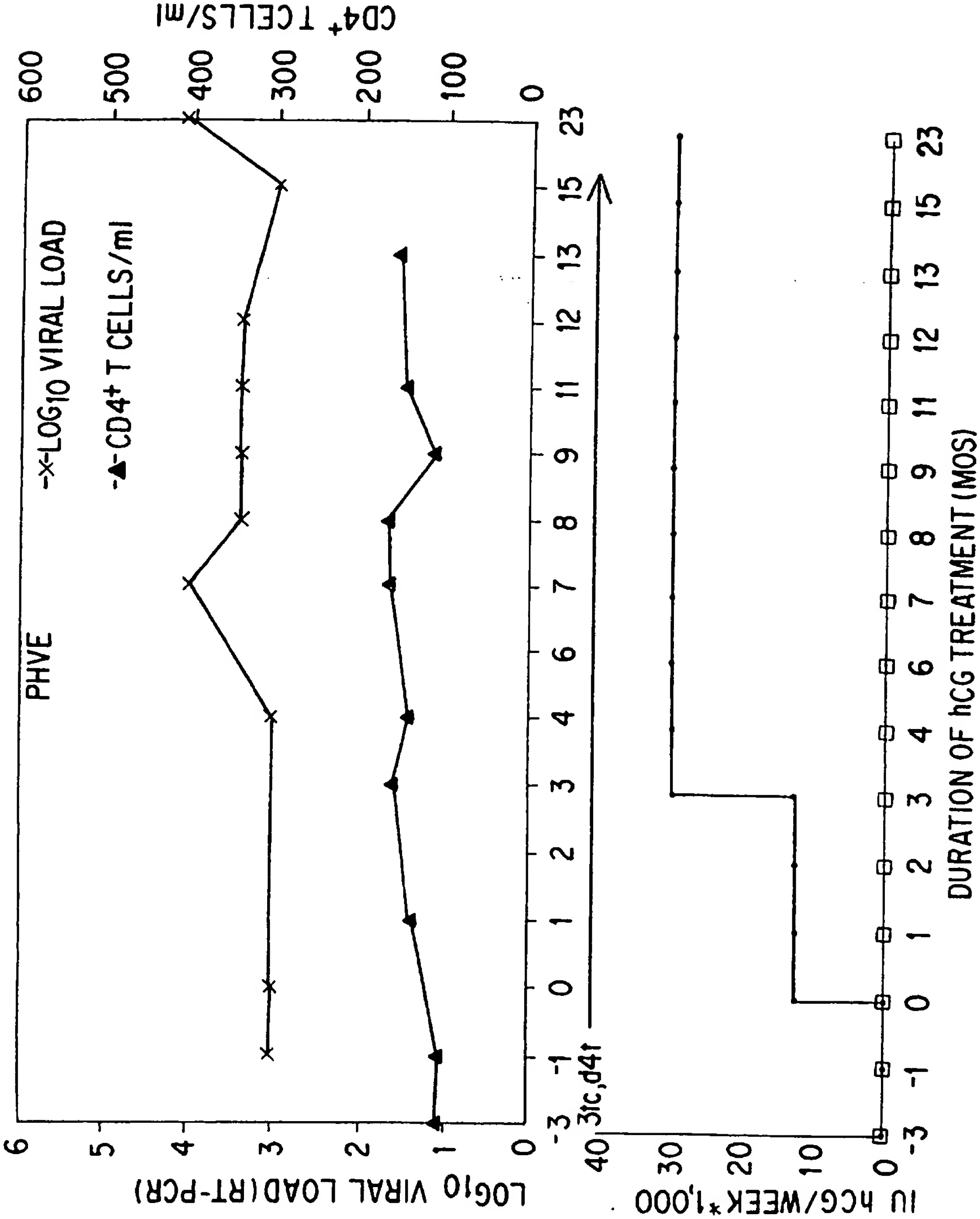

PHVE
-×-LOG10 VIRAL LOAD
-▲-CD4+ T CELLS/ml
LOG10 VIRAL LOAD (RT-PCR)
CD4+ T CELLS/ml
3tc,d4t
IU hCG/WEEK*1,000
DURATION OF hCG TREATMENT (MOS)

FIG.3G

FIG.3H

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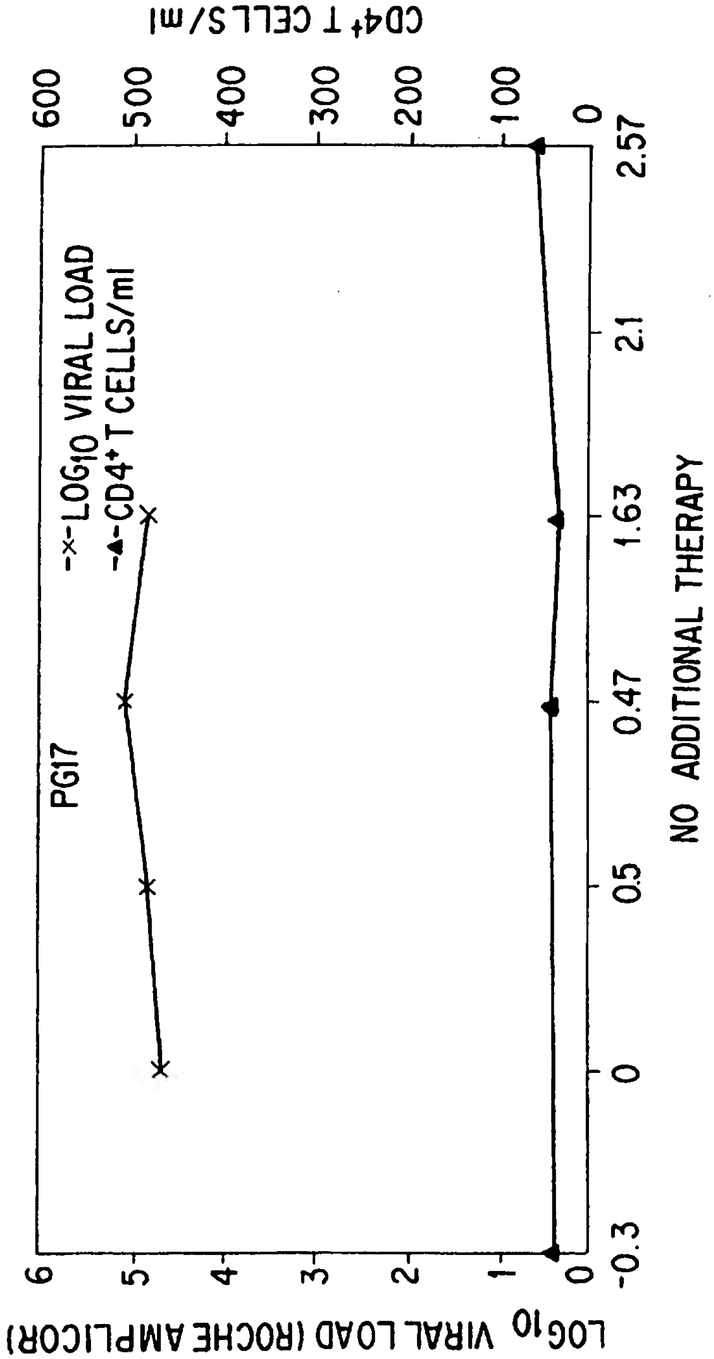


FIG.3I

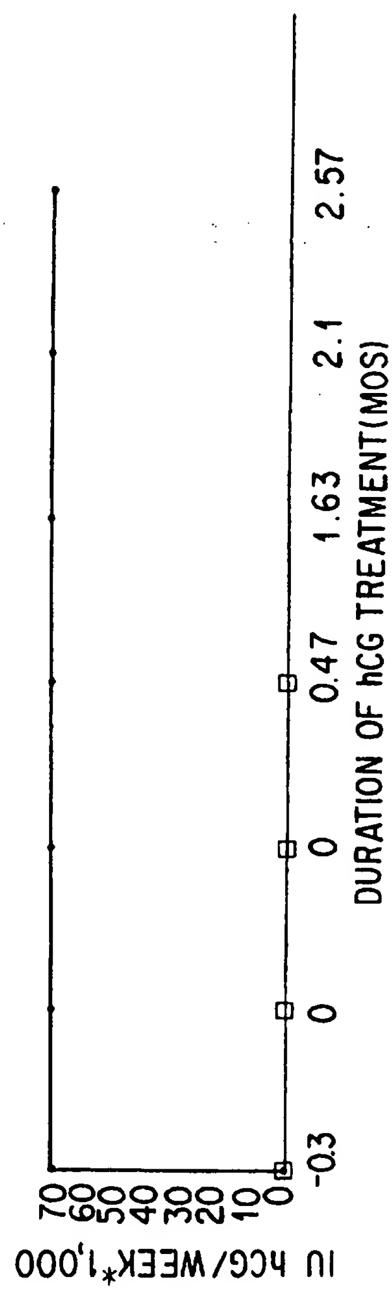


FIG.3J

```
AGACAAGGCA GGGGACGCAC CAAGG ATG GAG ATG TTC CAG GGG CTG CTG CTG    52
                            Met Glu Met Phe Gln Gly Leu Leu Leu
                            -20                 -15
TTG CTG CTG CTG AGC ATG GGC GGG ACA TGG GCA TCC AAG GAG CCG CTT   100
Leu Leu Leu Leu Ser Met Gly Gly Thr Trp Ala Ser Lys Glu Pro Leu
    -10                 -5                  1               5
CGG CCA CGG TGC CGC CCC ATC AAT GCC ACC CTG GCT GTG GAG AAG GAG   148
Arg Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu Ala Val Glu Lys Glu
                    10                  15                  20
GGC TGC CCC GTG TGC ATC ACC GTC AAC ACC ACC ATC TGT GCC GGC TAC   196
Gly Cys Pro Val Cys Ile Thr Val Asn Thr Thr Ile Cys Ala GLy Tyr
                25                  30                  35
TGC CCC ACC ATG ACC CGC GTG CTG CAG GGG GTC CTG CCG GCC CTG CCT   244
Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro
            40                  45                  50
CAG CTG GTG TGC AAC TAC CGC GAT GTG CGC TTC GAG TCC ATC CGG CTC   292
Gln Val Val Cys Asn Tyr Arg Asp Val Arg Phe Glu Ser Ile Arg Leu
        55                  60                  65
CCT GGC TGC CCG CGC GGC GTG AAC CCC GTG GTC TCC TAC GCC GTG GCT   340
Pro Gly Cys Pro Arg Gly Val Asn Pro Val Val Ser Tyr Ala Val Ala
70                  75                  80                  85
CTC AGC TGT CAA TGT GCA CTC TGC CGC CGC AGC ACC ACT GAC TGC GGG   388
Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg Ser THr Thr Asp Cys Gly
                    90                  95                  100
GGT CCC AAG GAC CAC CCC TTG ACC TGT GAT GAC CCC CGC TTC CAG GAC   436
Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp
                105                 110                 115
TCC TCT TCC TCA AAG GCC CCT CCC CCC AGC CTT CCA AGC CCA TCC CGA   484
Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg
            120                 125                 130
CTC CCG GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA TAAAGGCTTC        530
Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln
        135                 140                 145
TCAATCCGC                                                         539
```

FIG.4

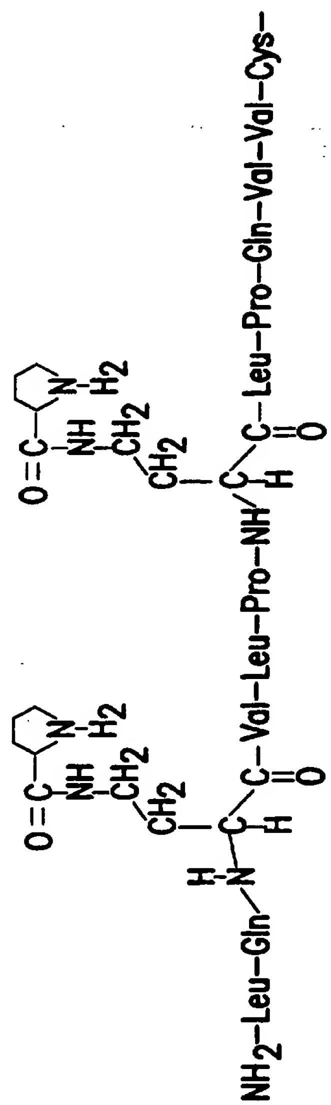


FIG.5A

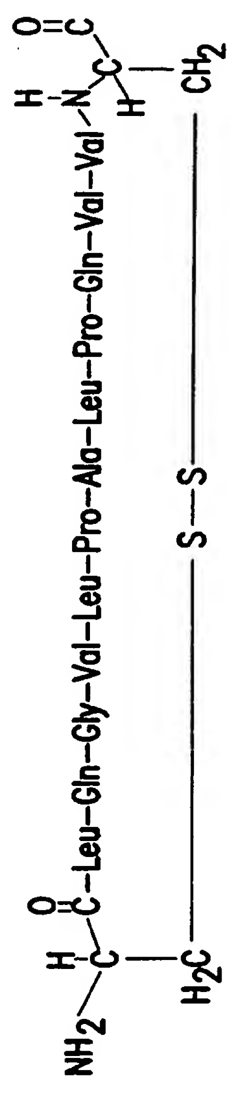


FIG.5B

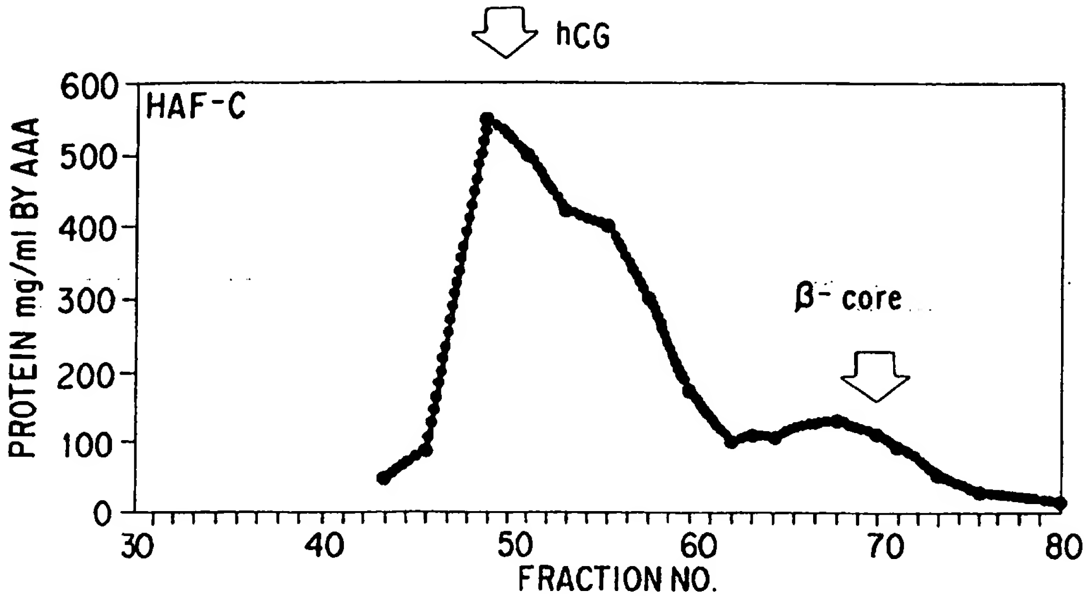

hCG
HAF-C
β- core
PROTEIN mg/ml BY AAA
600
500
400
300
200
100
0
30
40
50
60
70
80
FRACTION NO.

FIG. 6A

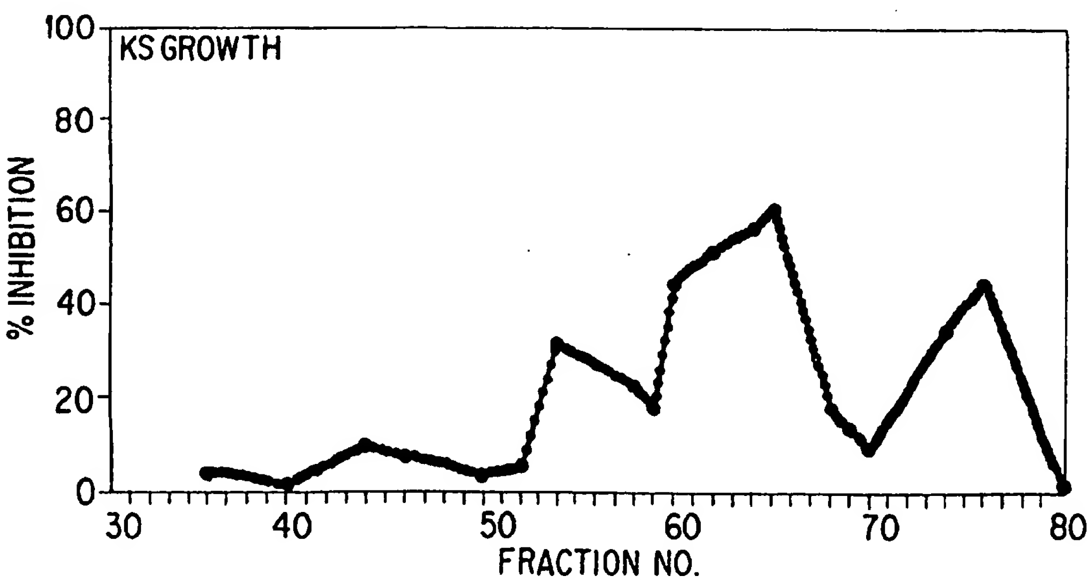

KS GROWTH
% INHIBITION
100
80
60
40
20
0
30
40
50
60
70
80
FRACTION NO.

FIG. 6B

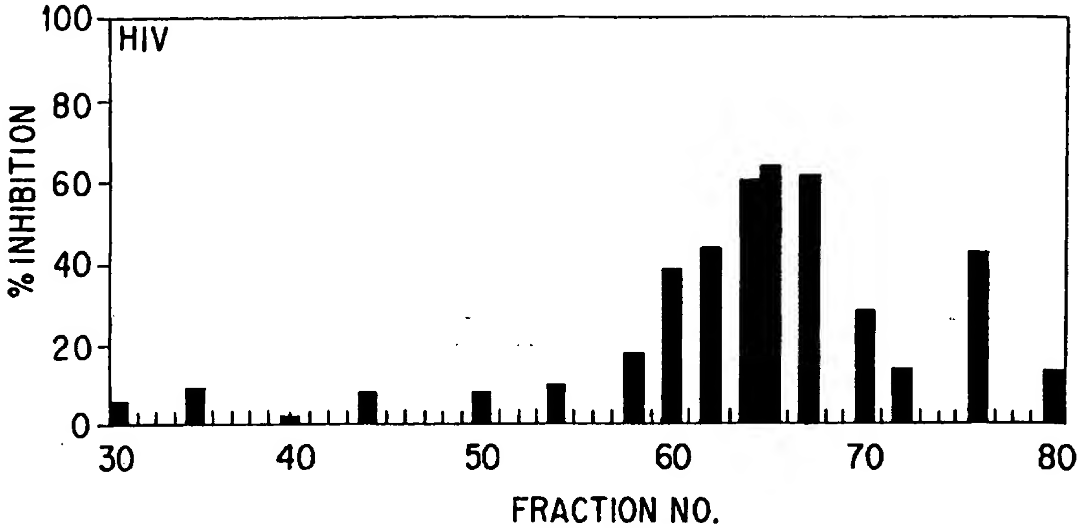

HIV
% INHIBITION
100
80
60
40
20
0
30
40
50
60
70
80
FRACTION NO.

FIG. 6C

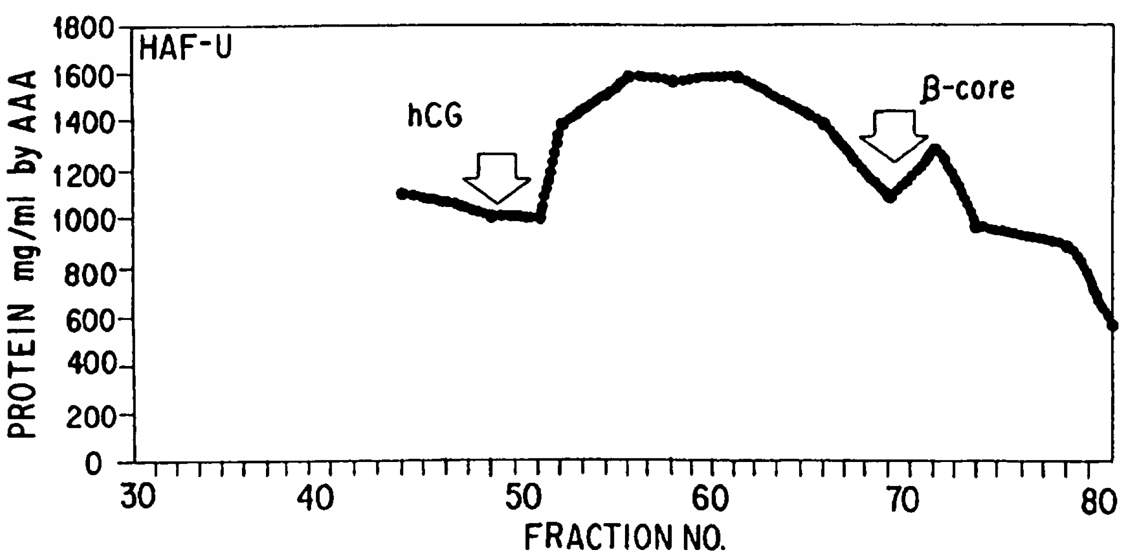

HAF-U
PROTEIN mg/ml by AAA
1800
1600
1400
1200
1000
800
600
400
200
0
hCG
β-core
30
40
50
60
70
80
FRACTION NO.

FIG. 6D

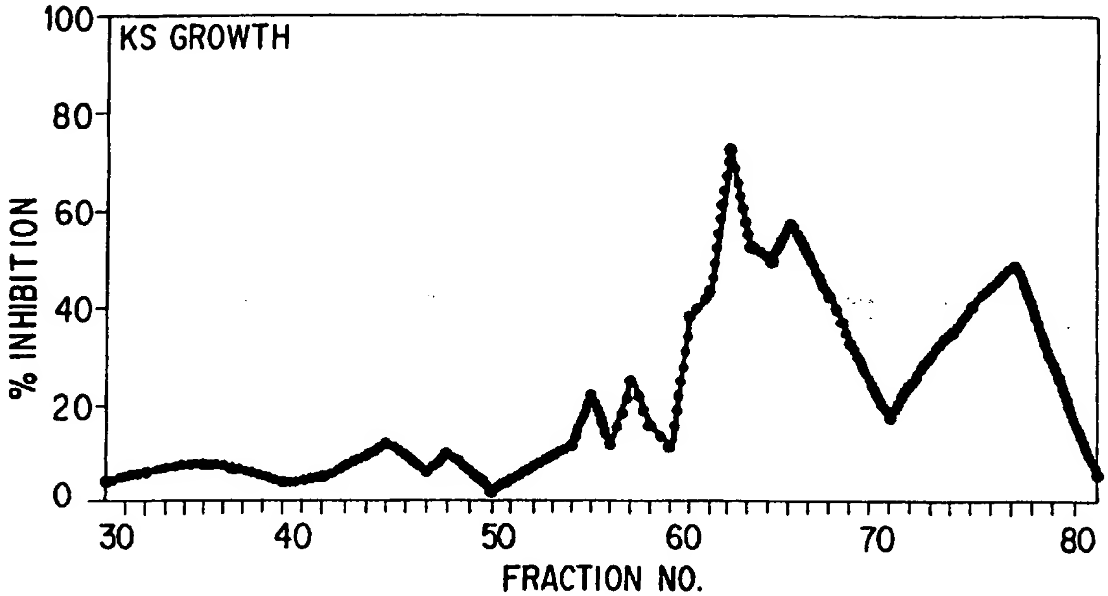

KS GROWTH
% INHIBITION
100
80
60
40
20
0
30
40
50
60
70
80
FRACTION NO.

FIG.6E

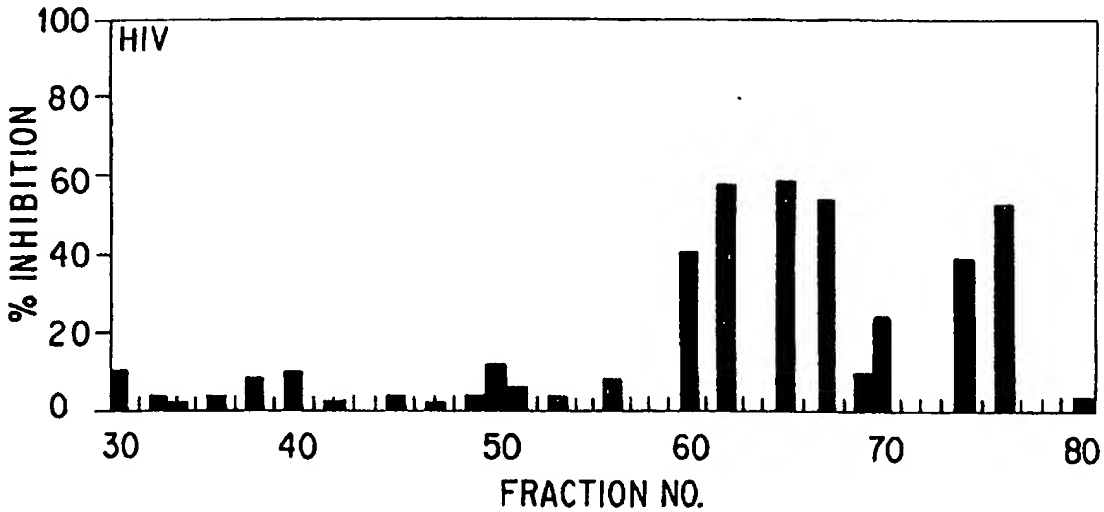
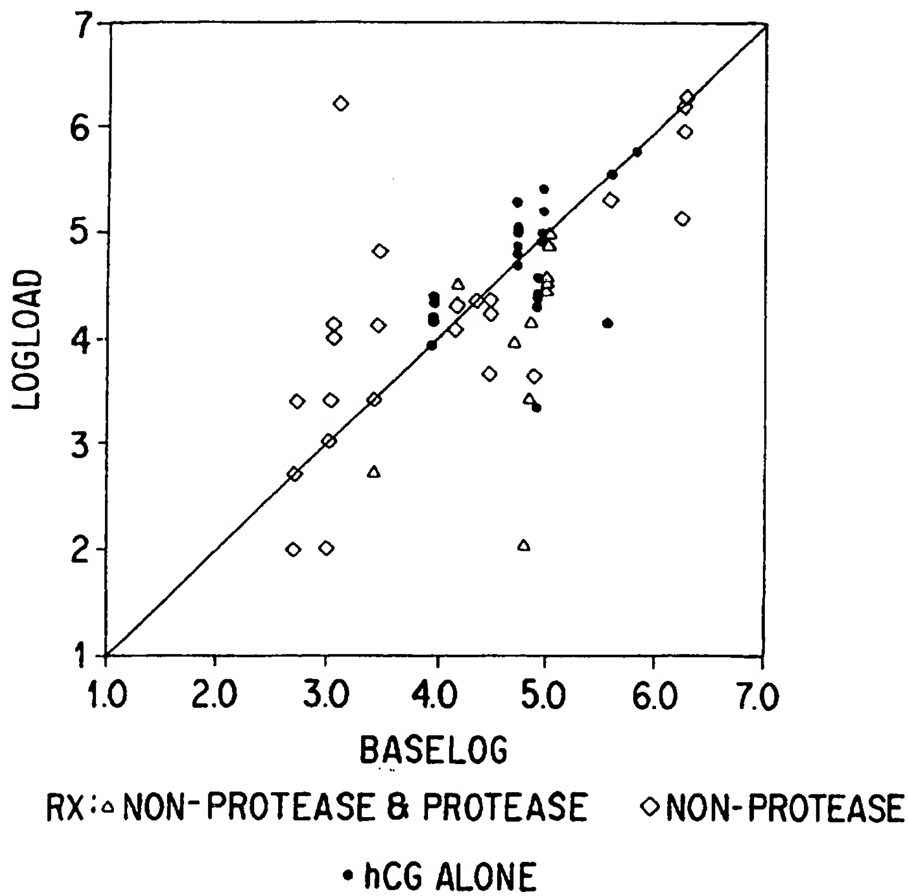
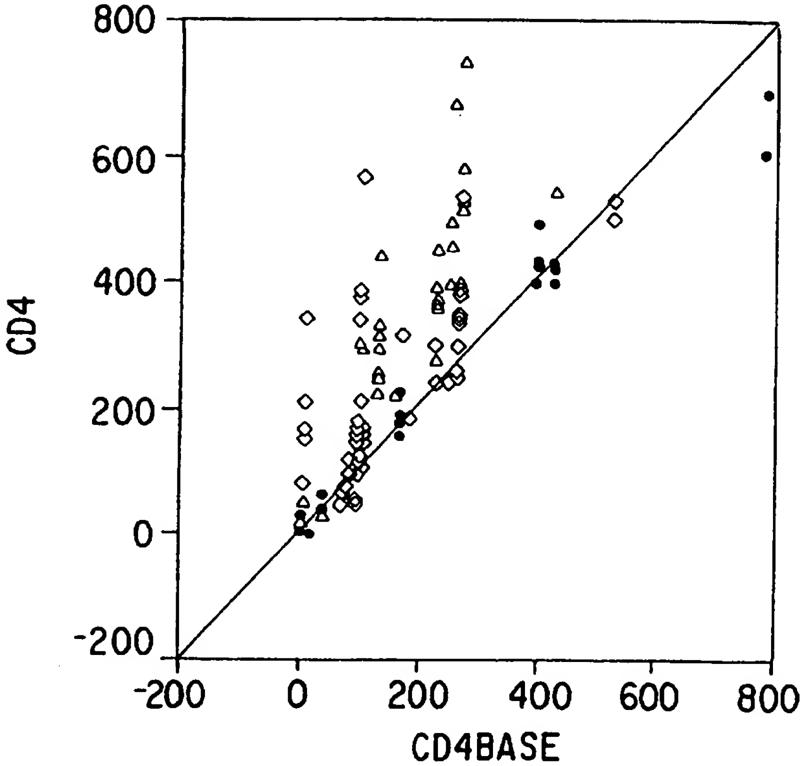

HIV
% INHIBITION
100
80
60
40
20
0
30
40
50
60
70
80
FRACTION NO.

FIG.6F



7
6
5
4
3
2
1
LOGLOAD
1.0
2.0
3.0
4.0
5.0
6.0
7.0
BASELOG
RX: △ NON-PROTEASE & PROTEASE ◇ NON-PROTEASE
• hCG ALONE

FIG.7A



RX: △ NON-PROTEASE & PROTEASE ◇ NON-PROTEASE

• hCG ALONE

FIG.7B

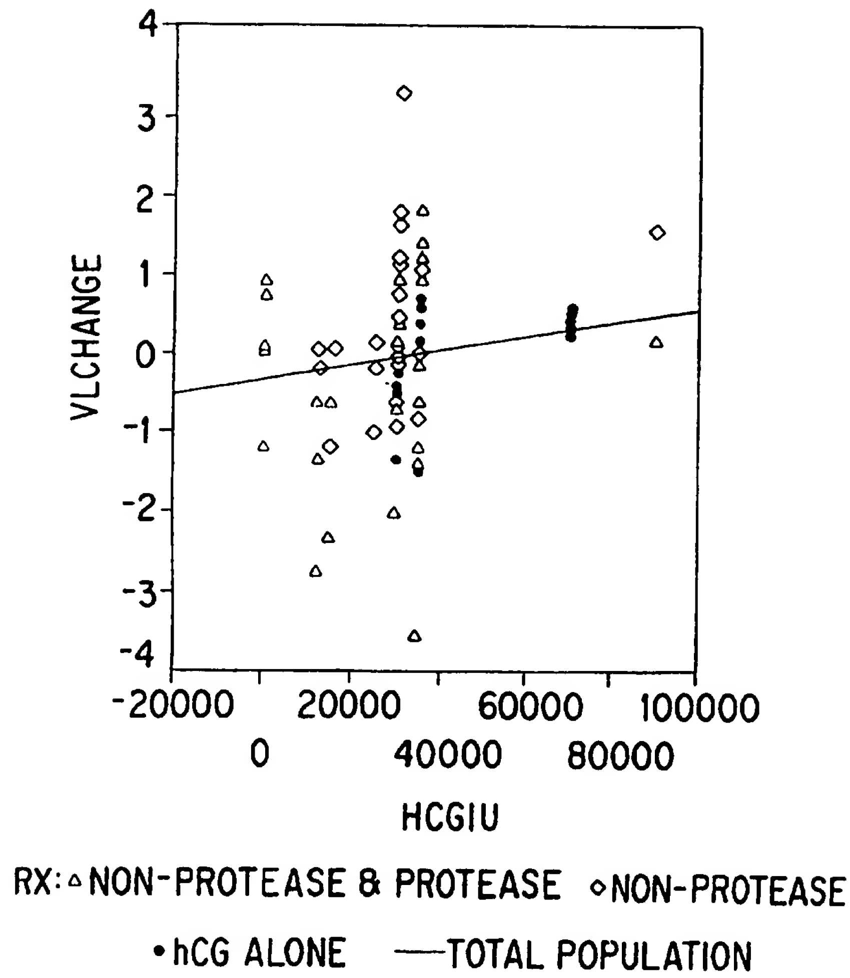

VLCHANGE
4
3
2
1
0
-1
-2
-3
-4
-20000
0
20000
40000
60000
80000
100000
HCGIU
RX: △ NON-PROTEASE & PROTEASE ◇ NON-PROTEASE
• hCG ALONE —TOTAL POPULATION

FIG.7C

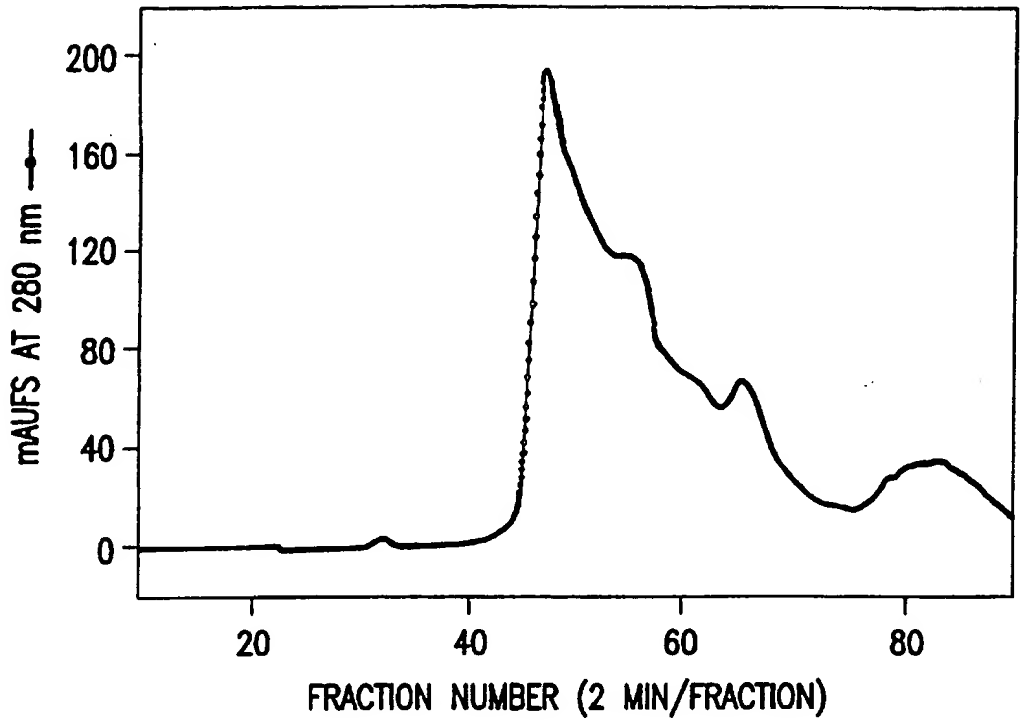

200
160
120
80
40
0
mAUFS AT 280 nm
20
40
60
80
FRACTION NUMBER (2 MIN/FRACTION)

FIG.8A

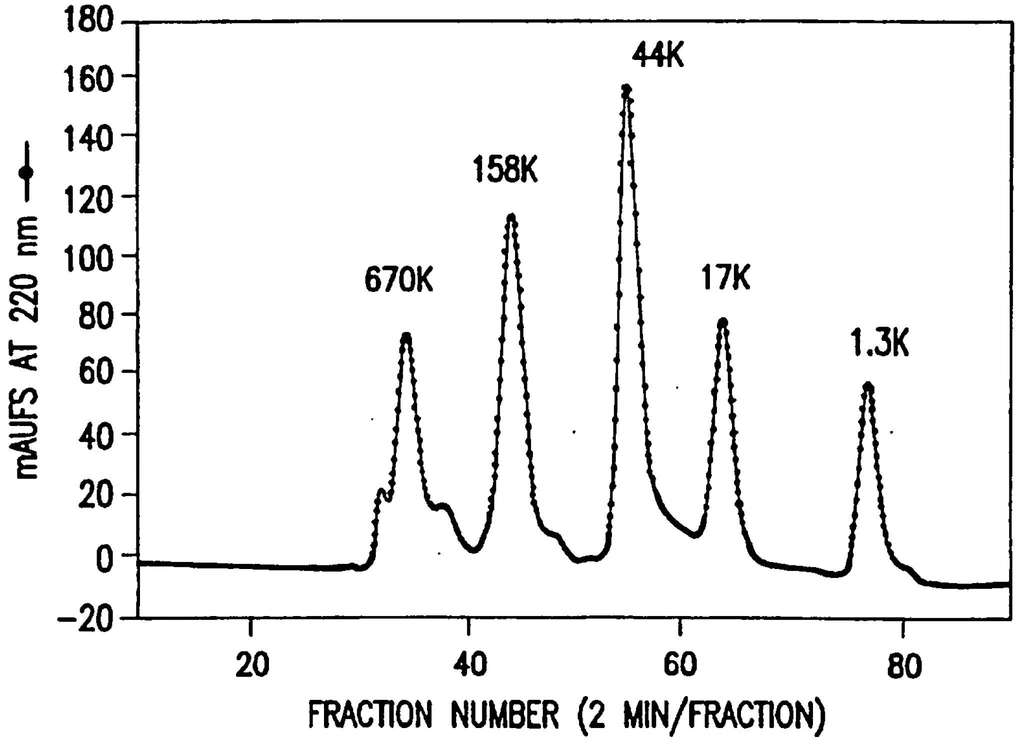

180
160
140
120
100
80
60
40
20
0
-20
mAUFS AT 220 nm
670K
158K
44K
17K
1.3K
20
40
60
80
FRACTION NUMBER (2 MIN/FRACTION)

FIG.8B

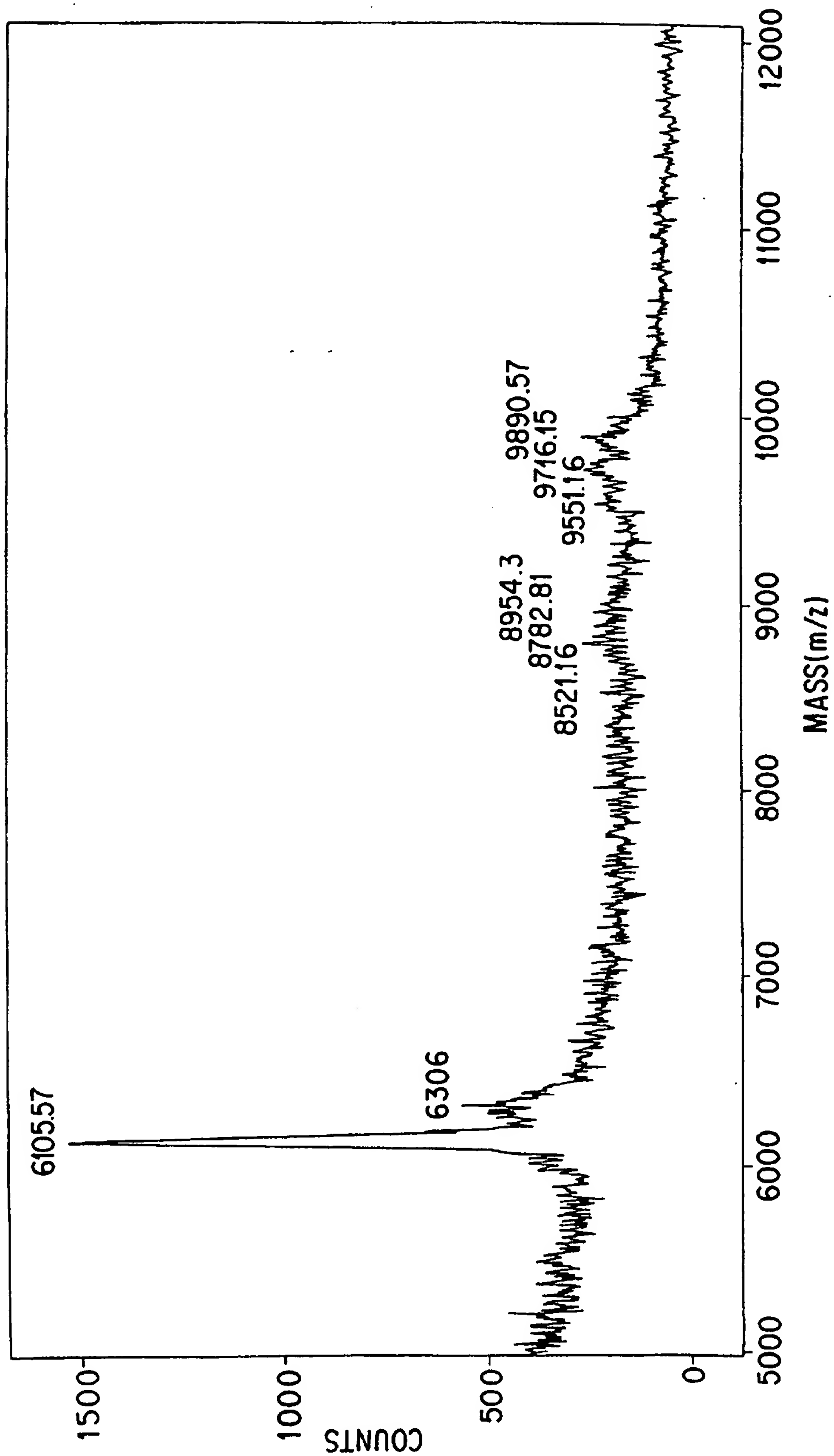

6105.57
6306
8954.3
8782.81
8521.16
9890.57
9716.15
9551.16
COUNTS
1500
1000
500
0
MASS(m/z)
5000
6000
7000
8000
9000
10000
11000
12000

FIG.9A

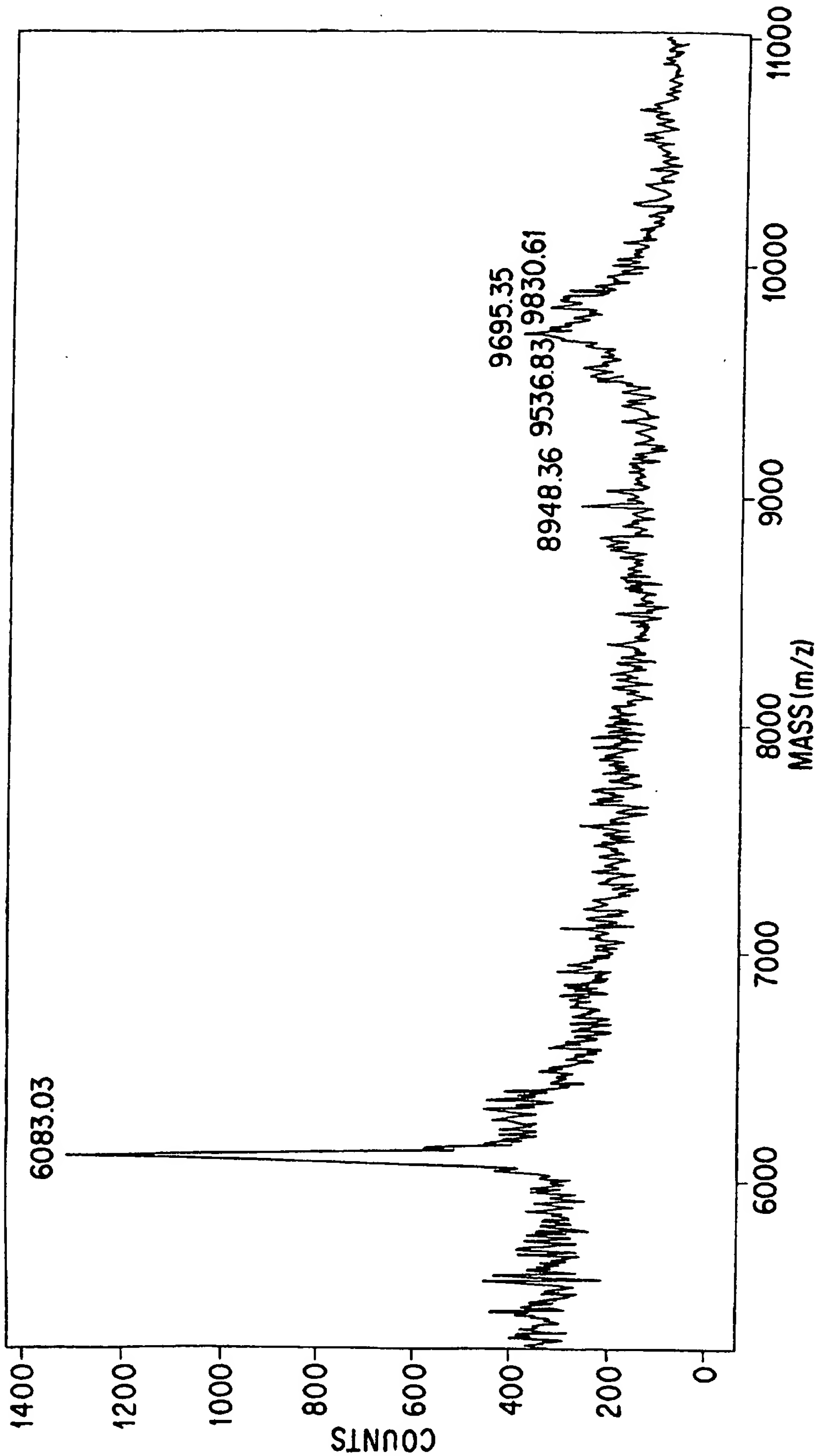

6083.03
8948.36
9536.83
9695.35
9830.61
COUNTS
0
200
400
600
800
1000
1200
1400
6000
7000
8000
9000
10000
11000
MASS (m/z)

FIG.9B

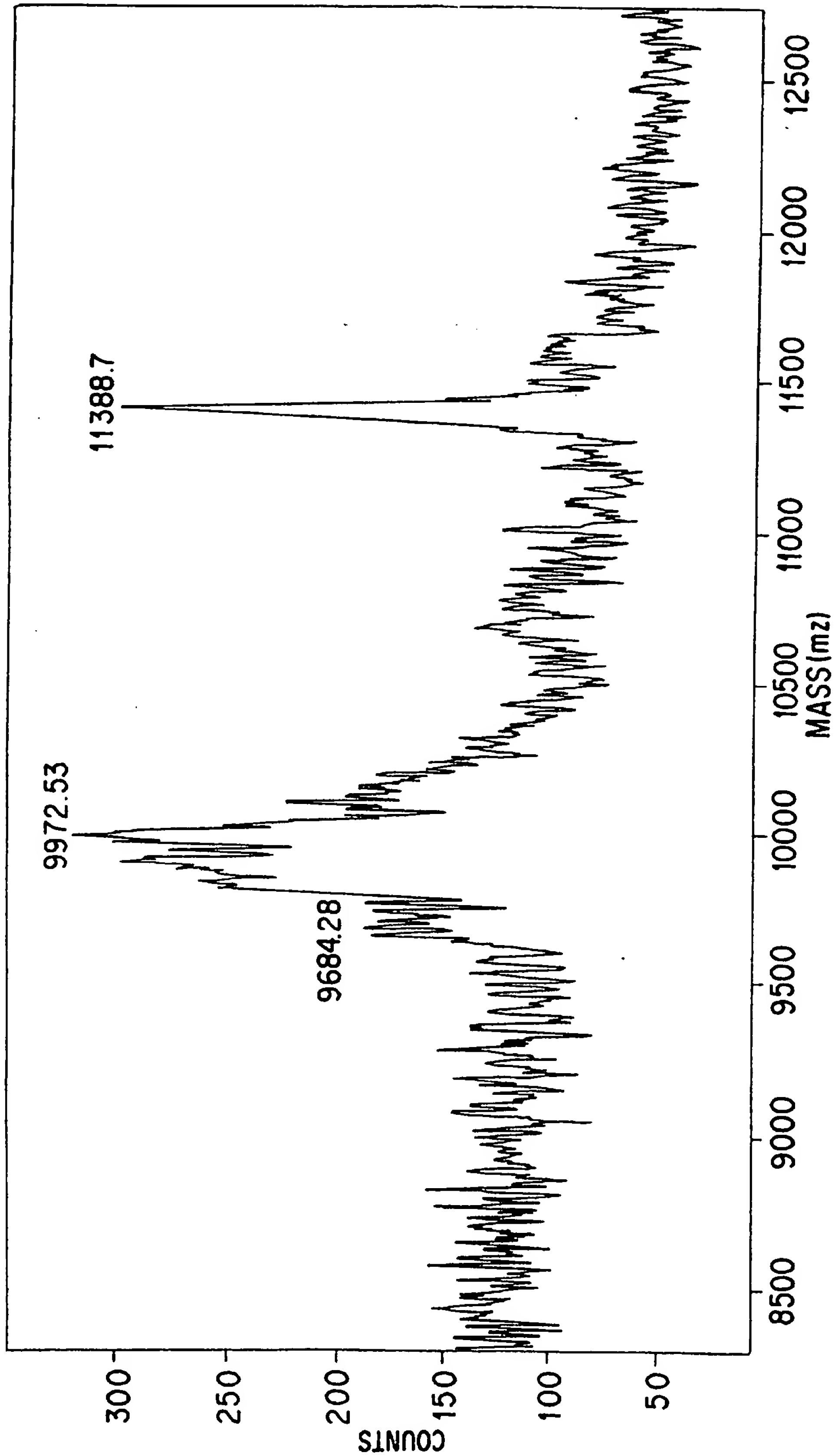

11388.7
9972.53
9684.28
8500
9000
9500
10000
10500
11000
11500
12000
12500
MASS(mz)
300
250
200
150
100
50
COUNTS

FIG.9C

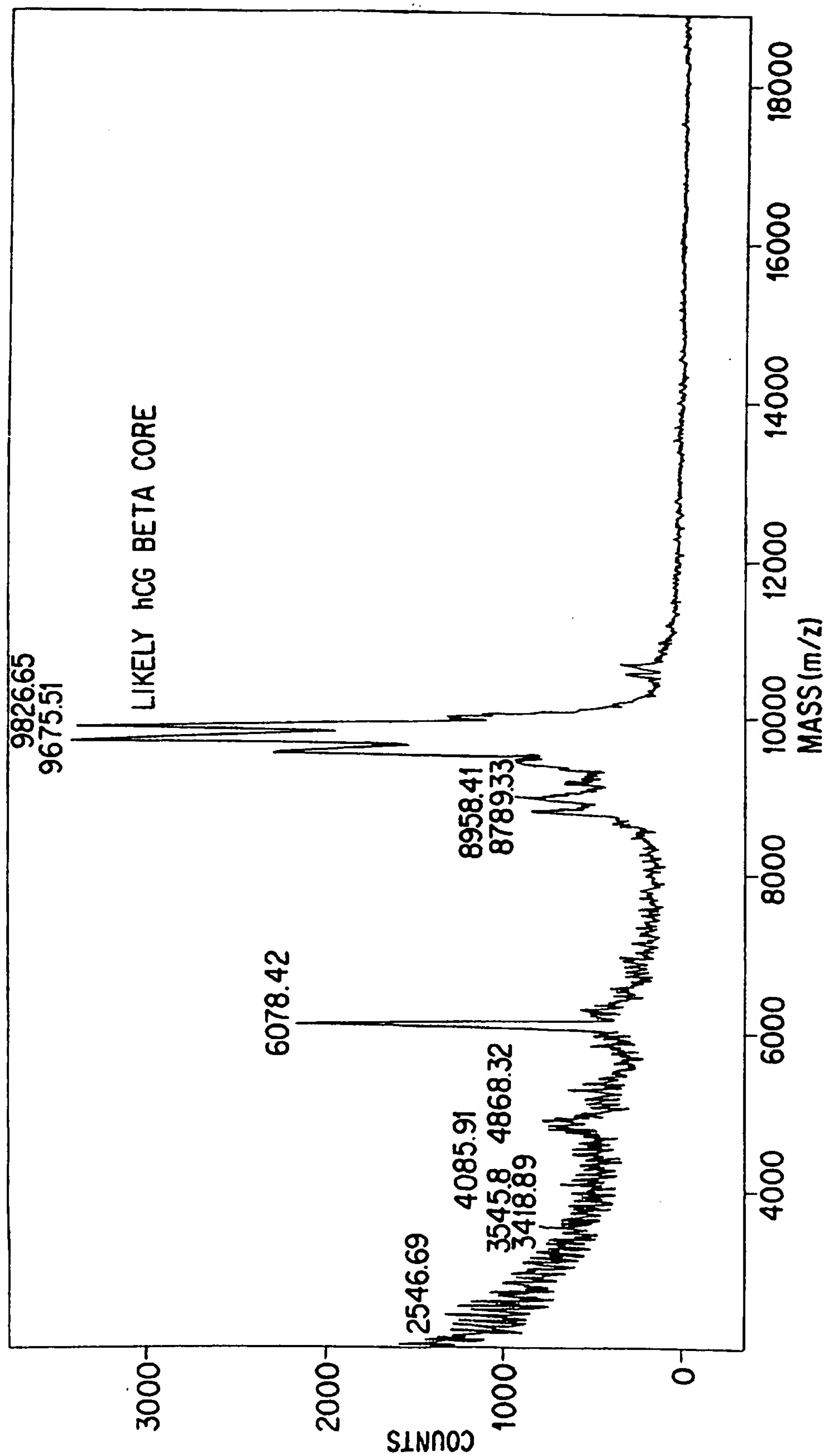

LIKELY hCG BETA CORE
9826.65
9675.51
8958.41
8789.33
6078.42
4868.32
4085.91
3545.8
3418.89
2546.69
COUNTS
0
1000
2000
3000
4000
6000
8000
10000
12000
14000
16000
18000
MASS (m/z)

FIG.9D

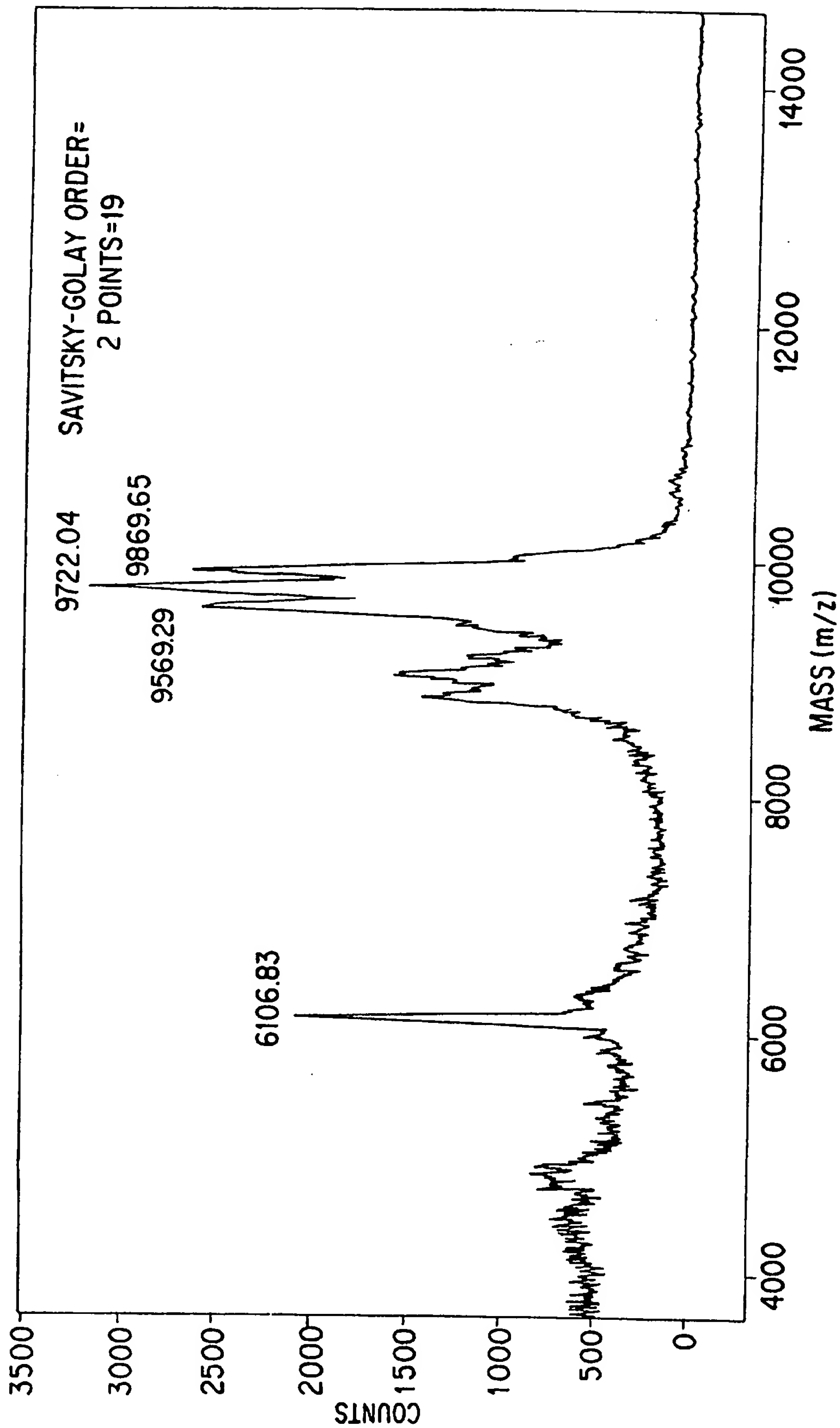

SAVITSKY-GOLAY ORDER=
2 POINTS=19
9722.04
9869.65
9569.29
6106.83
COUNTS
3500
3000
2500
2000
1500
1000
500
0
4000
6000
8000
10000
12000
14000
MASS (m/z)

FIG.9E

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/11448

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 5/00, 14/00; A61K 38/00
US CL :512/14; 530/350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 512/14; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, WPIDS, MEDLINE, DIALOG
search terms. human chorionic gonadotropin; hCG; wasting syndrome, HIV

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE et al. Human chorionic gonadotropin hormone prevents wasting syndrome and death in HIV-1 transgenic mice. Journal of Clinical Investigation. April 1997, Vol. 99, No. 7, pages 1484-1491.	1-6 and 49-50
X	LUNARDI-ISKANDAR et al. Tumorigenesis and metastasis of neoplastic Karposi's sarcoma cell line in immunodeficient mice blocked by a human pregnancy hormone. Nature. 04 May 1995, Vol. 375, pages 64-68. See pages 64-65.	1-6 and 49-50
X,P	PICARD et al. Preliminary results with human chorionic gonadotropin in AIDS-related Kaposi's sarcoma. Int. Conf. AIDS. Abstract No. Tu.B2227. August 1996, Vol. 11, No. 1, page 304. See Abstract.	1-6 and 49-50

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
23 SEPTEMBER 1997

Date of mailing of the international search report
12 NOV 1997

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
JULIE E. REEVES
Telephone No. (703) 308-0196

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LUNARDI-ISKANDAR et al. Human chorionic gonadotropin (hCG) induces apoptosis of neoplastic Kaposi's sarcoma cell lines (KS Y-1 and KS SLK). Blood. Abstract No. 1514. 1995, Vol. 86 (10 Suppl. 1). page 381A.	1-6 and 49-50
X,P	GILL et al. The effects of preparations of human chorionic gonadotropin on AIDS-related Kaposi's sarcoma. New England Journal of Medicine. 24 October 1996, Vol. 335, No. 17, pages 1261-1269, see entire document.	1-6 and 49-50.
A	RABKIN et al. Kaposi's sarcoma in pregnant women. Nature. 07 September 1995, Vol. 377, pages 21-22.	1-6 and 49-50

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/11448

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-6 and 49-50

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-6 and 49-50, drawn to a method of treating wasting syndrome by administering hCG or beta hCG and the pharmaceutical composition of hCG.
Group II, claim(s) 7-14 and 34-41 and Claims 19-20, in part, drawn to a method of treating wasting syndrome by administering hCG peptides. Group II will be searched to the extent that it reads upon hCG peptides that are not derivatized.
Group III, claim(s) 15-18, 21-33 and claims 19-20, in part, drawn to a method of treating wasting syndrome by administering derivatized hCG peptides. Group III will be searched to the extent that it reads upon hCG peptides that are derivatized.
Group IV, claim(s) 42-45, drawn to a method of treating wasting syndrome by administering mixtures of purified and unpurified hCG peptides.
Group V, claim(s) 46-48, drawn to a method of screening a preparation of hCG for anti-wasting activity.
Group VI, claim(s) 51-57, drawn to pharmaceutical compositions of hCG peptides.
Group VII, claim(s) 58-78, drawn to pharmaceutical compositions of derivatized hCG peptides.
Group VIII, claim(s) 79-85, drawn to pharmaceutical compositions containing mixtures of purified and unpurified hCG peptides.
Group IX, claim(s) 86-87, drawn to a method of treating wasting syndrome by the administration of nucleic acids encoding for hCG.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

If any of Groups II, III, VI or VII are elected, then an election of species is necessary between the 26 peptide sequences listed in claims 8, 16, 23, 28, 35, 51, 58, 64, 69, 76.

Species A:	SEQ ID NO: 3
Species B:	SEQ ID NO: 4
Species C:	SEQ ID NO: 5
Species D:	SEQ ID NO: 6
Species E:	SEQ ID NO: 7
Species F:	SEQ ID NO: 8
Species G:	SEQ ID NO: 9
Species H:	SEQ ID NO: 10
Species I:	SEQ ID NO: 11
Species J:	SEQ ID NO: 12
Species K:	SEQ ID NO: 13
Species L:	SEQ ID NO: 14
Species M:	SEQ ID NO: 15
Species N:	SEQ ID NO: 16
Species O:	SEQ ID NO: 17
Species P:	SEQ ID NO: 18
Species Q:	SEQ ID NO: 19
Species R:	SEQ ID NO: 20
Species S:	SEQ ID NO: 21
Species T:	SEQ ID NO: 22
Species U:	SEQ ID NO: 23
Species V:	SEQ ID NO: 24
Species W:	SEQ ID NO: 25
Species X:	SEQ ID NO: 33
Species Y:	SEQ ID NO: 34
Species Z:	SEQ ID NO: 35

The inventions listed as Groups I-IX do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I, VI, VII and VIII recite pharmaceutical compositions comprising hCG, portions of hCG, derivatized portions of hCG and mixtures of purified and unpurified hCG, respectively. Each of these pharmaceutical compositions contain the special technical feature of proteins with different amino acid sequences which would result in peptides with different biological, physiological and immunological properties, such as different anti-wasting activities, different peptide stabilities and different antigenic properties. For the mixtures of purified and unpurified hCG, Group VIII contains impurities and heterologous species that are not recited in any of Groups I, VI or VII.

Groups I, II, III, IV recite methods of treating by administering hCG, portions of hCG, derivatized portions of hCG and mixtures of purified and unpurified hCG, respectively. Each of these pharmaceutical compositions contain the special technical feature of proteins with different amino acid sequences which would result in peptides with different biological, physiological and immunological properties, such as different anti-wasting activities, different peptide stabilities and different antigenic properties. For the mixtures of purified and unpurified hCG, Group VIII contains impurities and heterologous species that are not recited in any of Groups I, II or III. Administering thee different compounds would result in different pharmacological effects.

Groups I-IV differ from Groups V and IX, because Groups I-IV recite the administration of a peptide for treatment, while Group V recites a method of screening for anti-wasting activity while group IX recites a method of administering nucleic acid. Thus the special technical features of a method of protein treatment, a method of gene therapy and a method of screening are not linked under PCT Rule 13.2.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: Species A-Z are not linked under PCT Rule 13.1 because they each recite the special technical feature of a different amino acid sequence which would result in peptides with different biological, physiological and immunological properties, such as different anti-wasting activities, different peptide stabilities and different antigenic properties.

If any of Groups II, III, VI or VII are elected, then the first ten peptide sequences (Species A through Species J, SEQ ID NOs 3-12, respectively) will be searched if no additionally fees are paid.